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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 2021

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Report of statistical information on the use of animals in procedures

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I. Abstract

To improve the accuracy of reporting and to allow data interrogation at Member State-level, the Directive was revised in 2019 through Regulation (EU) 2019/1010[1]. In 2020, Commission Implementing Decision 2020/569/EU[2] was adopted to accommodate the new obligations including an introduction of some new reporting categories to break down categories with a significant number of miscellaneous entries. The new categories took effect for data of 2021 and are explicitly mentioned throughout this report, leading to a decrease of uses reported under the "Other" categories.

In 2021, after three years of consecutive decrease, the total number of animals, individuals used for the first time, increased significantly to 9.41 million (+18.5% compared to 2020). This increase is mainly due to three projects (two using salmon in Norway and one using larval form of sea bass in Spain) accounting for over 1.3 million animals which represents 14% of total uses in 2021. As these projects concerned animals used for animal diseases and disorders, animal welfare and animal nutrition, the use category translational and applied research increased by +53.9% and became the first purpose for animal used for research and testing. Part of the increase can also be attributed to projects that were either cancelled or postponed in 2020 due to COVID-19 lockdowns and carried out in 2021. Another part of the increase in the category translational and applied research is also related to a reporting of uses for a new category, animal nutrition, that were previously reported under basic research that decreases consequently by -8.2%. Meanwhile, the number of uses of animals used for regulatory purposes continued to decrease for the fourth consecutive year (-6.2%).

For the first time, the content of the different sections considers the evolution of uses of animals over time, comparing the 2021 data with 2020 and using the year 2018 as reference to identify longer term trends.

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

1 OJ L 170, 25.6.2019, p. 115–127

2 OJ L 129, 24.4.2020, p. 16–50

3 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 2021 under Directive 2010/63/EU[4] ("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 since 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 the 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. For the first time, the content of the different sections considers the evolution of uses of animals over time, comparing the 2021 data with 2020 and using the year 2018 as reference to identify longer term trends.

To improve the accuracy of reporting and to allow data interrogation at Member State-level, the Directive was revised in 2019 through Regulation (EU) 2019/1010[5]. In 2020, Commission Implementing Decision 2020/569/EU[6] was adopted to accommodate the new obligations including an introduction of some new reporting categories to break down categories with a significant number of miscellaneous entries. The new categories took effect for data of 2021 and are explicitly mentioned throughout this report.

The data presented in this report can now be consulted at both Union and Member State level using the open-access public ALURES Statistical EU database[7].

II.1. Numbers and origins of animals

In 2021, the total number of animals, individuals used for the first time, in research and testing covering the 28 countries (EU-27 and Norway) was just above 9.41 million. This is +18,5% higher than in 2020 but limited to +6.6% compared to 2018 (Table 1).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Total	8,822,404	8,579,439	7,938,064	**9,406,233**	+18.5%	+6.6%

Table 1: Total numbers of animals used for the first time between 2018 and 2021

4 Directive 2010/63/EU OJ L276, 20.10.2010, p.33-79

5 OJ L 170, 25.6.2019, p. 115–127

6 OJ L 129, 24.4.2020, p. 16–50

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

Analysing the increase in more detail, three projects stand out, two using salmon in Norway and one using larval form of sea bass in Spain as mentioned in their respective Member State narratives. These three projects which account for over 1.3 million animals and represent almost 14% of all animals used for the first time in 2021 in the Union. Previously these would have been reported under category "Other fish" but thanks to the 2020 amendment to the species categories, they can now be identified separately (Figure 1). Part of the increase can also be attributed to projects that were either cancelled or postponed in 2020 due to COVID-19 lockdowns and carried out in 2021. At the same time, 13 Member States had a reduction in the number of animals used for the first time in 2021 compared to 2020.

Looking at species used (Figure 1), 2021 saw an important increase of the proportion of fish (36.5%) used for the first time for the above-mentioned reason, mainly salmon, trout, chars and graylings (20.4%) and see bass (6.9%). Against this, the proportion of mice decreased (43.5%), however, still remaining as the most used species, followed by rats (7.3%), domestic fowls (4.4%) and rabbits (4%).

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 2021, the proportion of animals born outside of the Union represented only 2.2% of all animals used for the first time (excluding non-human primates) (Section IV.1.2). The proportion of animals born in the Union but not at a registered breeder increased, representing 17% 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 2021, 5,314 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 breeding purposes, 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.

For the first time, this report includes a separation of the generation of the non-human primates from the type of colony (self-sustaining or not) they originated. In 2021, 58.8% of them came from self-sustaining colonies (Section IV.1.2).

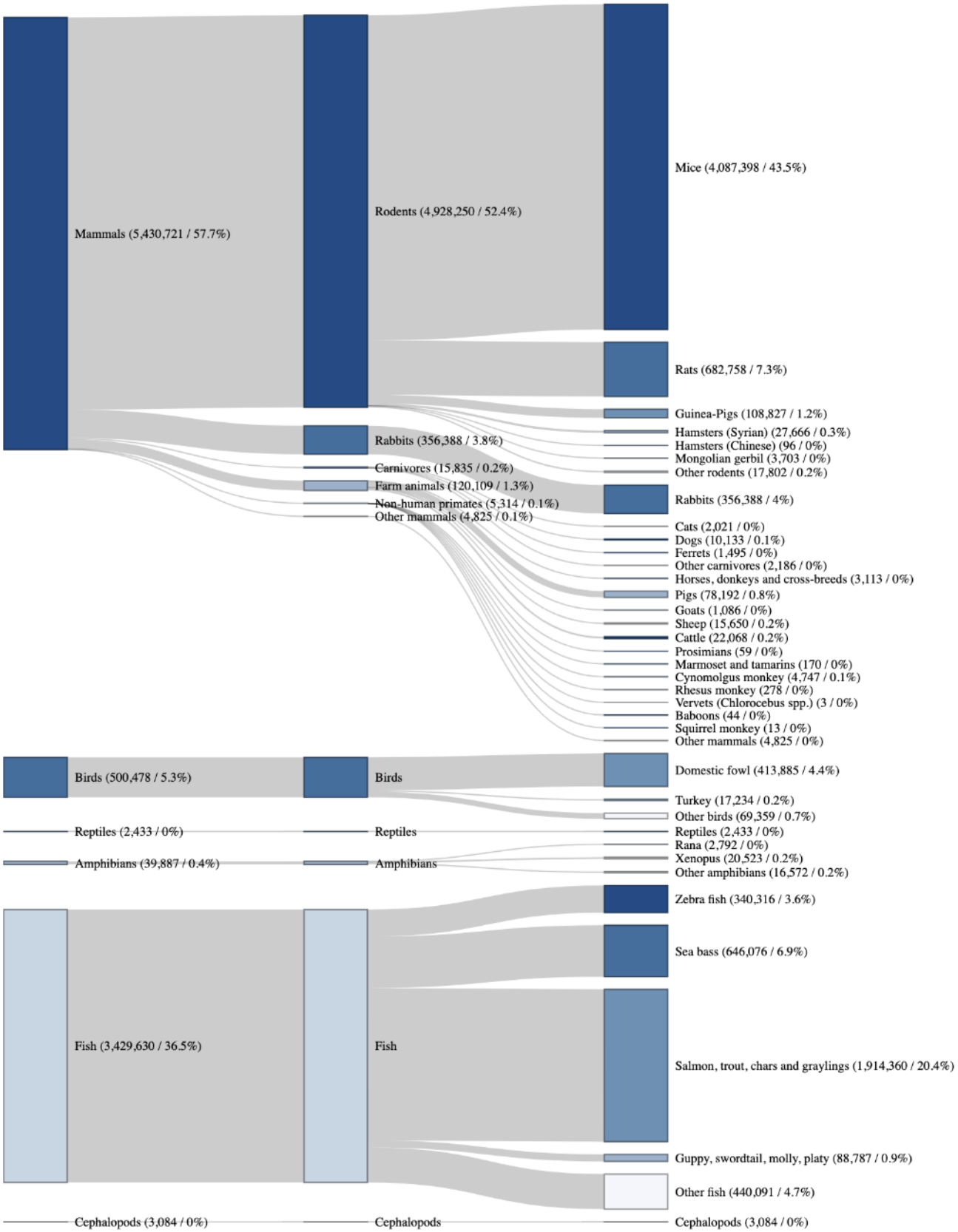


Figure 1: Numbers of animals used for the first time by main classes of species in 2021

II.2. Uses of animals in research and testing

In 2021, 9.51 million uses (including first and any subsequent reuse) of animals for scientific purposes were reported (Figure 2). As in previous years, the main purpose was research (73%) but for the first time, the number of uses for translational and applied research purposes (41%) exceeded the number of uses for basic research (32%). A further 14% of animal uses were for regulatory use to satisfy regulatory requirements, followed by other uses (9%) and routine production (4%).

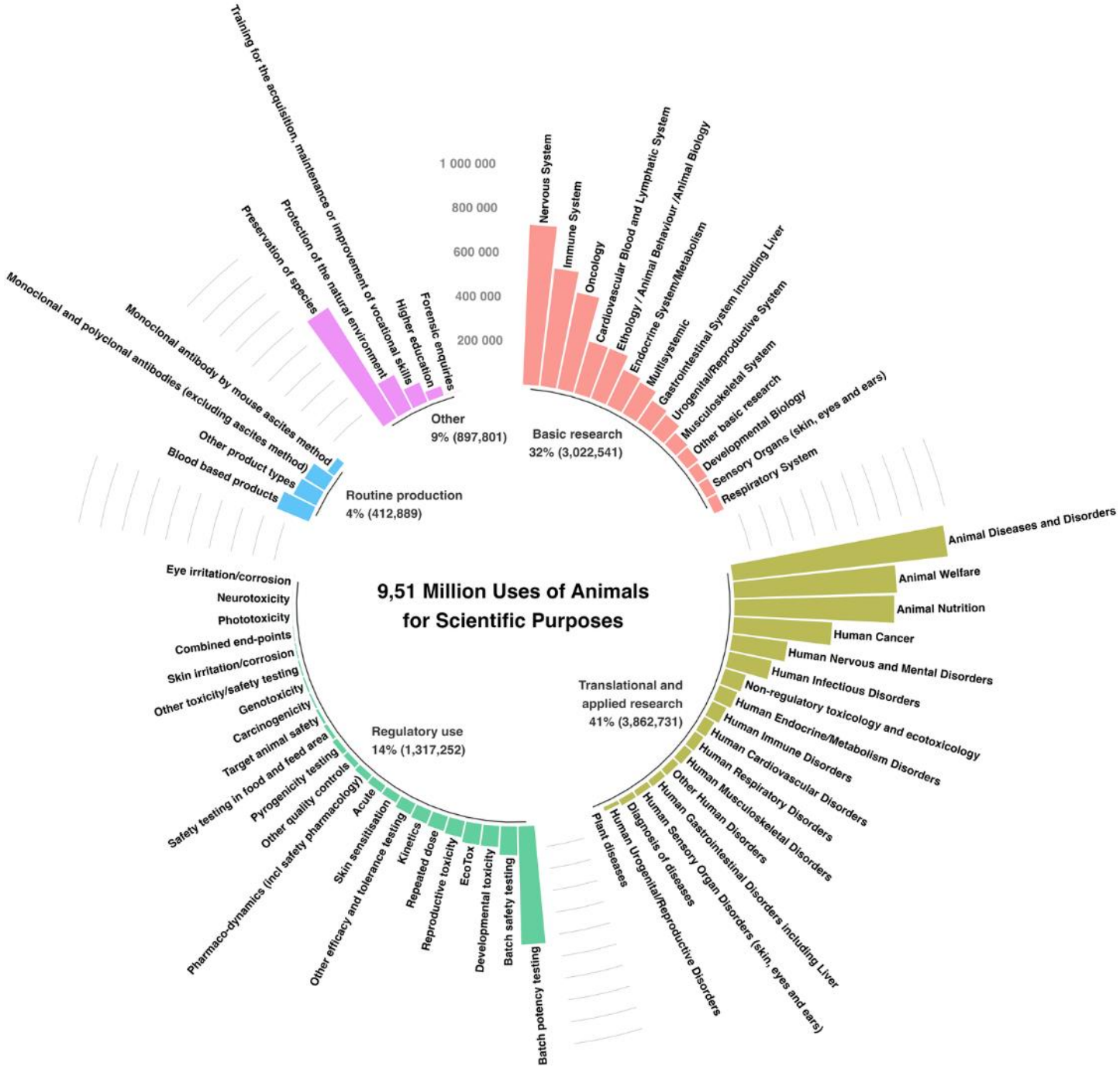


Figure 2: All uses of animals for research and testing in 2021

In terms of evolution, uses for translational and applied research increased the most (+53.9%) compared to 2020. This increase can be explained by the three projects led by Norway and Spain on fish mentioned above that belong to this category. Uses for the protection of species increased also significantly (+452.1%) explained by Norway in the Member State narrative as a correction of previously wrong reporting in this category.

Basic research decreased (-8.2%), a change that can be explained by the introduction of new reporting categories and which resulted in a significant decrease (-63%) of uses reported under "Other basic research". It would seem that categories developmental biology under basic research and animal nutrition under applied research have received the vast majority of entries previously reported under this category. Lastly, regulatory uses continue to decrease (-6.2%) while routine uses remained almost stable (+1.5%) (Section IV.2.1).

The proportion of 'severe' and 'non-recovery' uses continued a downward trend, decreasing a further with -0.2 and -0.6 percentage points respectively.

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

Table 2: Severity of uses reported in 2021

The graphical presentation in Figure 3 shows the purpose areas with most severe uses. In 2021, 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.

When analysing all the sub-categories of purposes, animal disease and disorders is now the highest number of severe uses (about 183,876 uses) followed by batch potency testing (134,502). Looking at the proportion of severe uses within a sub-category: production of monoclonal antibodies by mouse ascites is the first (54.6%), followed by neurotoxicity (40.5%) and acute toxicity (38.6%).

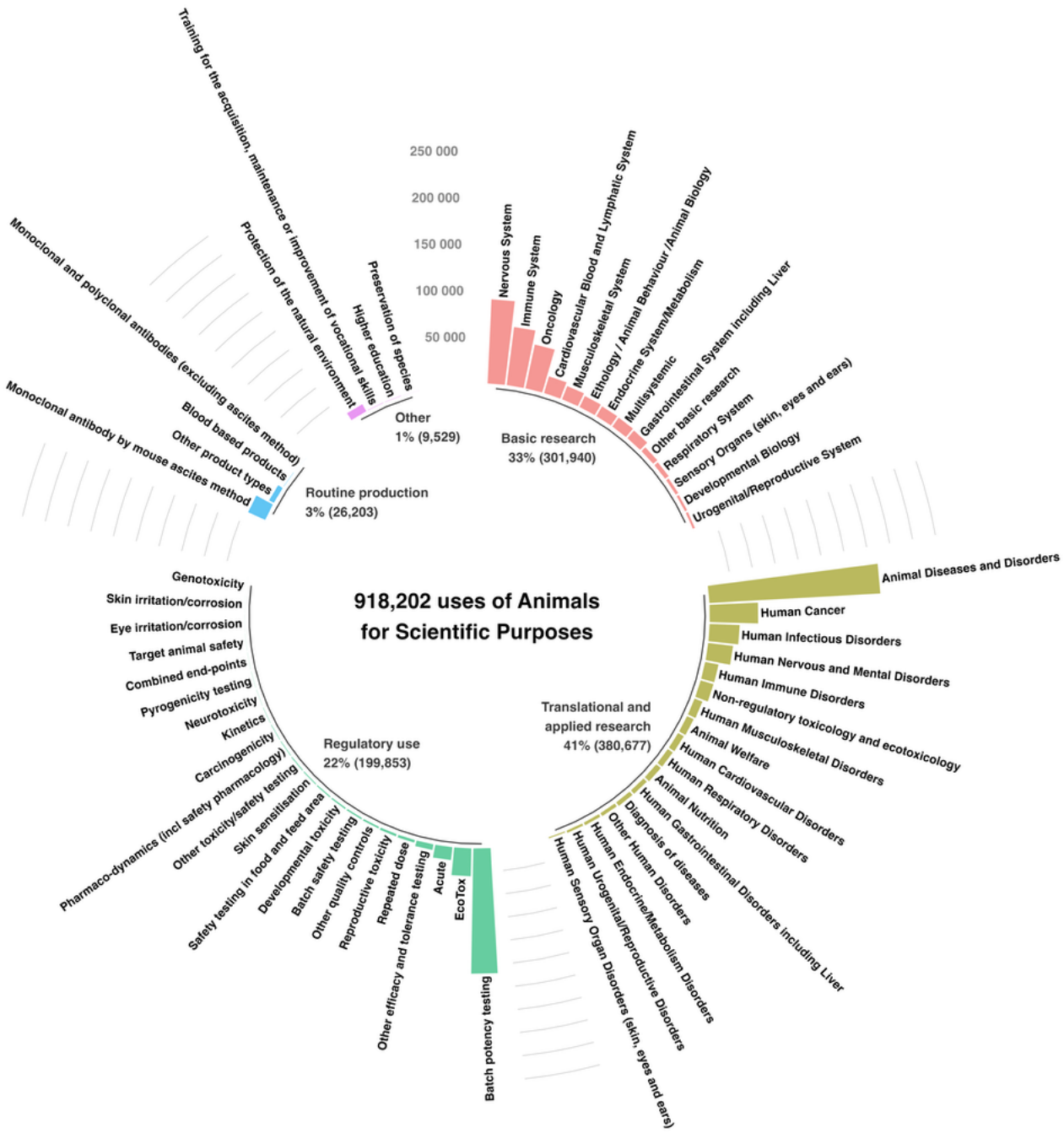


Figure 3: Severe uses of animals for research and testing in 2021

In 2021, 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 (51.1%), veterinary medicinal products (22.9%) and industrial chemicals (12.5%) (Figure 4).

Most of regulatory uses continue to be performed to comply with regulatory requirements originating from the Union legislation (96.2%). In terms of severity level resulting from uses to satisfy regulatory requirements, 15.2% of these uses were reported as severe, 22.9% as moderate, 61.2% mild (and up to mild) and 0.7% as non-recovery (Section IV.2.2.2.1).

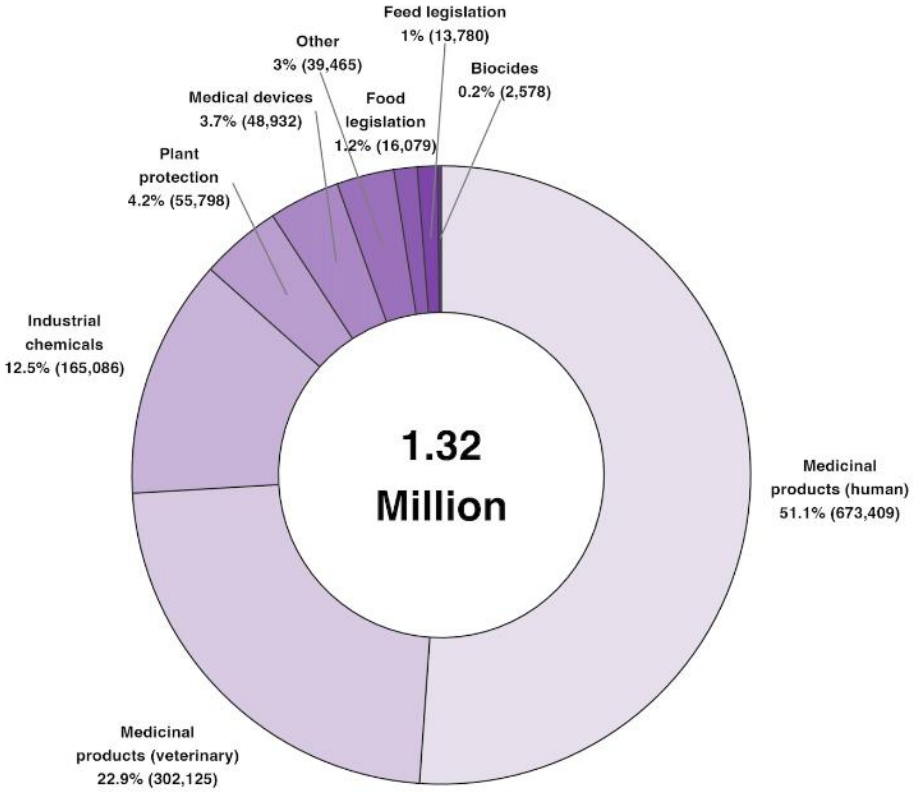


Figure 4: Regulatory uses by type of legislation in 2021

In line with the principle of the Three Rs[8], 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 slightly decreased and is now around 1.1% of all uses (compared to 1.7% in 2018). Proportionally, large mammals are reused more often, such as farm animals, cats, dogs, and some species of non-human primates.

Another novelty in the reporting categories is the separation of higher education from training for the acquisition, maintenance or improvement of vocational skills. It is now possible to detect that the highest proportion of reuses (10.7%) is for the purposes of higher education (Section IV.2.3).

The proportion of genetically altered animals used in research and testing remained stable compared to years 2018 to 2020. In 2021, 1.95 million uses were carried out on animals that were genetically altered. 3.5% had a harmful phenotypic alteration and 17% a non-harmful phenotype (Figure 5). Zebra fish and mice continue to be the most genetically altered species with 49.2% and 41.7% of all uses respectively.

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

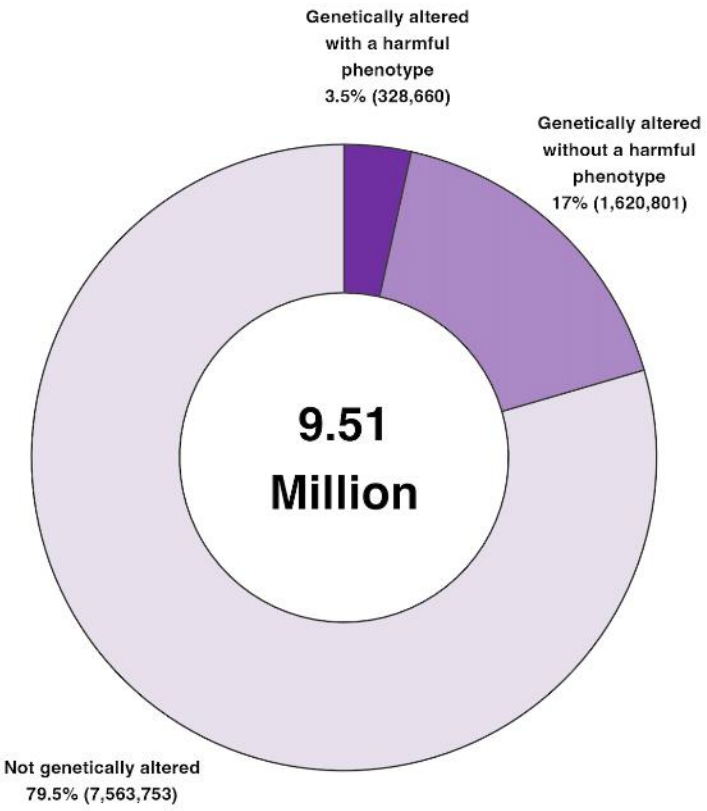


Figure 5: Genetic status of animals used in 2021

Genetically altered animals are used almost exclusively for research purposes with basic research accounting for 73.5% 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 664,965. There was an overall decrease of -3.2%, driven mainly by the creation of new genetically altered animal lines (-8.2%) while the maintenance of existing GA lines showing an increase of 3.5% compared to 2020 (Table 3).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
GA Creation	378,876	311,714	388,729	**356,706**	-8.2%	-5.9%
GA Maintenance	531,068	347,055	297,899	**308,259**	+3.5%	-42%
Total	**909,944**	**658,769**	**686,628**	**664,965**	**-3.2%**	**-26.9%**

Table 3: Total numbers of animals used for the creation and maintenance of genetically altered animal lines in 2021

In 2021, the number of animal uses for the creation of new genetically altered animal lines remained stable at 356,706 uses compared to previous years. The main species used for this purpose were, as before, mice and zebra fish, 71% and 26.1% respectively (Section IV.3).

In 2021, the category basic research covered 95.2% of all uses for the creation of new genetically altered animal lines. The purposes for which these were created are: multisystemic research (29.7%), cardiovascular, blood and lymphatic system (13.5%) and nervous system (13.4%).

Animals used for the first time for the 'maintenance of colonies of genetically altered animals of established lines' remained stable since 2019. The guidance document on genetically altered animals under the Directive[9] was endorsed by Member States National Contact Points responsible for the implementation of the Directive at their meeting in November 2021 and it is expected to improve harmonisation and accuracy of reporting of genetically altered animals.

9 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[10] 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 2021 data was laid out in Commission Implementing Decision 2020/5697/EU[11].

This statistical report contains the results of the data collected by all 27 Member States and Norway in 2021. 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

For the first time, 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, and replacing Decision 2012/707/EU[12].

All submitted data is available at open access ALURES Statistical EU Database[13] 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 2021.

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 2021. Numbers from previous years (2018-2020) have been recalculated by excluding data from the United Kingdom. For the first time, a systematic trend analysis provides information on the evolution of the Directive's objectives, this is done by comparing 2021 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 States 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

10 Directive 2010/63/EU OJ L276, 20.10.2010, p.33-79

11 OJ L 129, 24.4.2020, p. 16–50

12 OJ L 320, 17.11.2012, p. 33–50

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

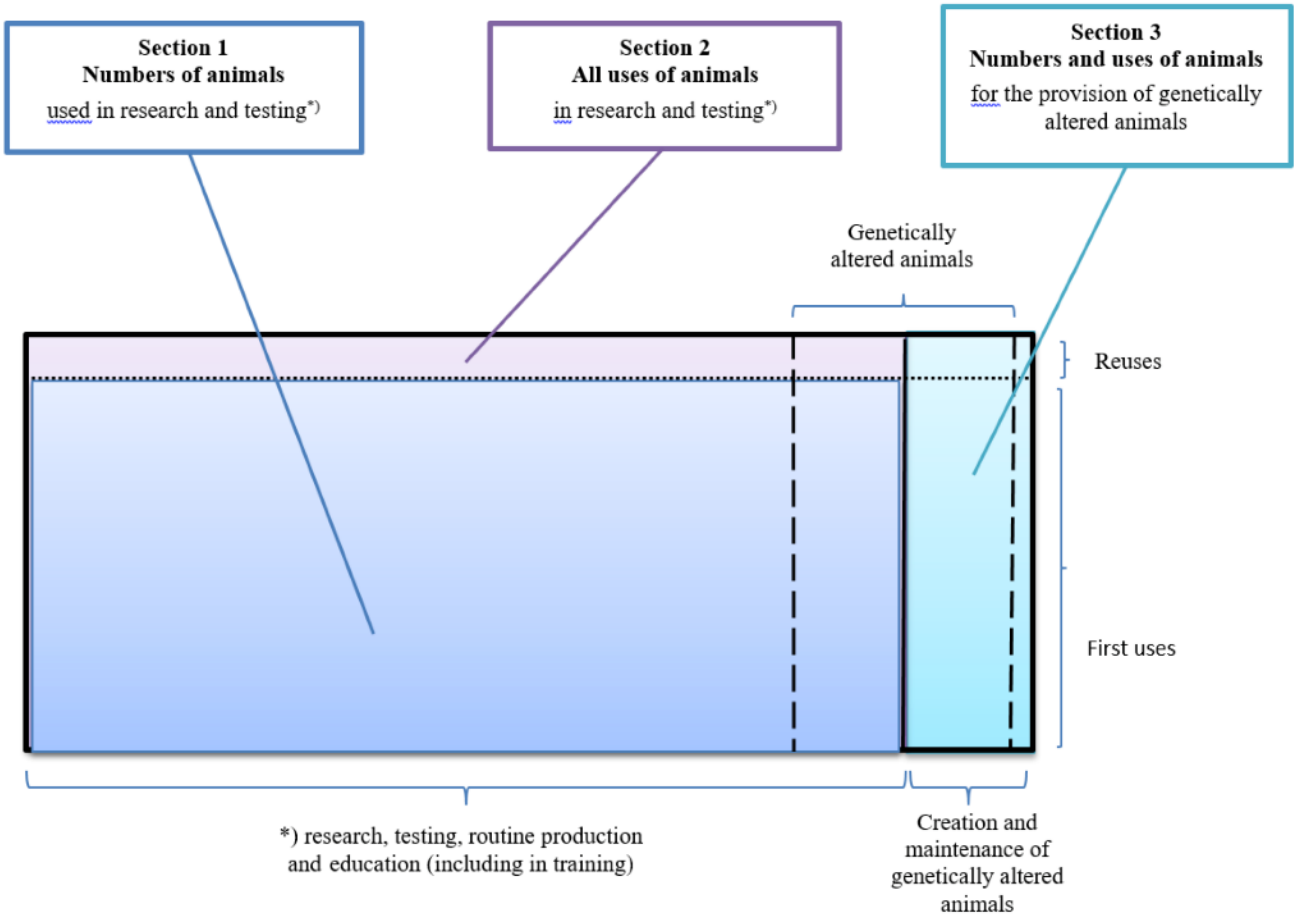
the content of 'other' categories (for example, "Other rodents", "Other basic research") or for understanding the origin of projects using particularly high numbers of animals.

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[14] 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;
- 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.

[14] 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.

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).

Creation

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".

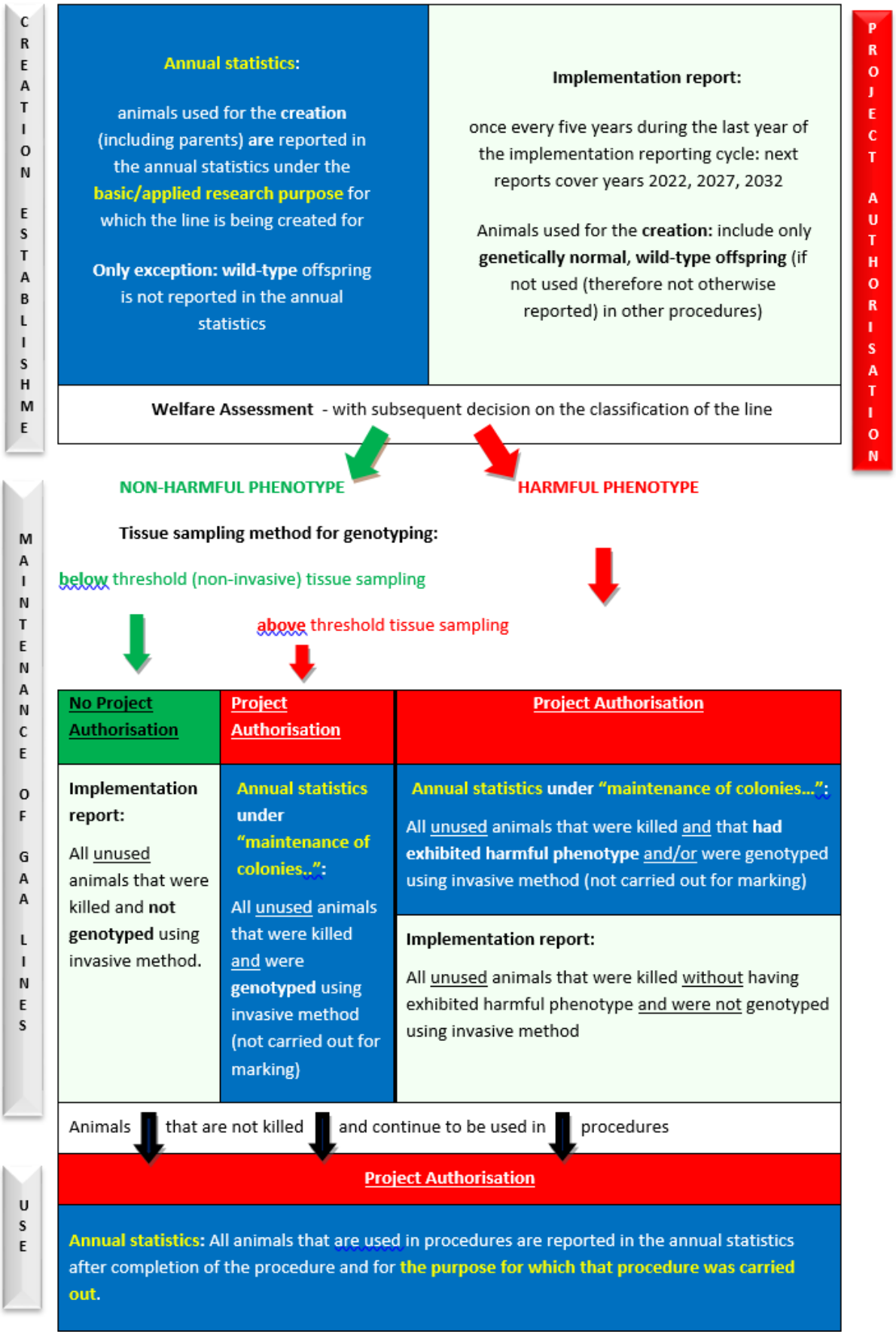
The welfare assessment determines if the newly established line is expected to have an *intended harmful phenotype (characteristic/trait)* i.e., an effect of genetic alteration that impacts negatively on an animal's health or welfare, such as muscle weakness, diabetes, or tumor development.

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 2021

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 2021, the number of animals used for the first time in the Union is 9.41 million, including the data from Norway and without the United Kingdom.

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Mammals	5,885,013 (66.7%)	5,751,005 (67%)	5,195,063 (65.4%)	**5,430,721 (57.7%)**	+4.5%	-7.7%
Birds	436,316 (4.9%)	498,218 (5.8%)	510,108 (6.4%)	**500,478 (5.3%)**	-1.9%	+14.7%
Reptiles	1,544 (0%)	1,972 (0%)	2,072 (0%)	**2,433 (0%)**	+17.4%	+57.6%
Amphibians	24,412 (0.3%)	43,499 (0.5%)	37,821 (0.5%)	**39,887 (0.4%)**	+5.5%	+63.4%
Fish	2,470,851 (28%)	2,267,777 (26.4%)	2,191,367 (27.6%)	**3,429,630 (36.5%)**	+56.5%	+38.8%
Cephalopods	4,268 (0%)	16,968 (0.2%)	1,633 (0%)	**3,084 (0%)**	+88.9%	-27.7%
Total	**8,822,404 (100%)**	**8,579,439 (100%)**	**7,938,064 (100%)**	**9,406,233 (100%)**	**+18.5%**	**+6.6%**

Table 4: Numbers of animals used for the first time by main types of species between 2018 and 2021

Table 4 above shows an increase of +18.5% compared to 2020, but the increase is only +6.6% compared to 2018 data. Looking at the main categories of species, this is mainly due to an important increase of fish uses (+56.5% compared to 2018 and +38.8% compared to 2018).

The proportional distribution of species used for the first time evolved significantly compared to 2020 with an increase of the proportion of fish (+8.9%) and a decrease of mammals (-7.7%), while other categories remained stable in proportion (table 4). Table 5 shows the breakdown of species used for the first time in 2021 by types of species. Mice are the most used species (43.5%), followed by salmon, trout, char and graylings (20.4%), rats (7.3%), sea bass (6.9%), domestic fowl (4.4%) and rabbits (4%) (Figure 1).

Fish used for the first time knew an important increase in 2021 (+56.5%). Numbers of fish tend to be higher than other species as studies involving fish larvae can concern tens of thousands of animals. This is particularly true for 2021 reported data as only three projects account for about 1.3 million uses of fish.

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Mammals						
Rodents						
Mice	4,410,737	4,318,913	3,879,691	**4,087,398**	+5.4%	-7.3%
Rats	829,906	792,744	665,155	**682,758**	+2.6%	-17.7%
Guinea-Pigs	123,486	111,652	111,172	**108,827**	-2.1%	-11.9%
Hamsters (Syrian)	9,397	10,427	17,355	**27,666**	+59.4%	+194.4%
Hamsters (Chinese)	20	17	149	**96**	-35.6%	+380%
Mongolian gerbil	4,269	3,672	2,978	**3,703**	+24.3%	-13.3%
Other rodents	19,534	28,273	28,186	**17,802**	-36.8%	-8.9%
Rabbits						
Rabbits	332,097	342,644	343,521	**356,388**	+3.7%	+7.3%
Carnivores						
Cats	1,517	2,140	2,464	**2,021**	-18%	+33.2%
Dogs	14,802	10,388	8,716	**10,133**	+16.3%	-31.5%
Ferrets	1,041	1,455	1,250	**1,495**	+19.6%	+43.6%
Other carnivores	4,267	4,325	6,867	**2,186**	-68.2%	-48.8%
Farm animals						
Horses, donkeys and cross-breeds	1,626	1,388	3,831	**3,113**	-18.7%	+91.5%
Pigs	79,699	77,424	73,509	**78,192**	+6.4%	-1.9%
Goats	1,443	1,124	998	**1,086**	+8.8%	-24.7%
Sheep	17,398	15,782	17,489	**15,650**	-10.5%	-10%
Cattle	22,580	19,059	22,175	**22,068**	-0.5%	-2.3%
Non-human primates						
Prosimians	170	194	54	**59**	+9.3%	-65.3%
Marmoset and tamarins	289	142	196	**170**	-13.3%	-41.2%
Cynomolgus monkey	5,349	4,741	4,220	**4,747**	+12.5%	-11.3%
Rhesus monkey	210	182	227	**278**	+22.5%	+32.4%
Vervets (Chlorocebus spp.)	16	25	34	**3**	-91.2%	-81.2%
Baboons	30	33	53	**44**	-17%	+46.7%
Squirrel monkey	25	0	0	**13**	NA	-48%
Other species of Old World Monkeys (Cercopithecoidea)	22	2	0	**0**	NA	-100%
Other mammals						
Other mammals	5,083	4,259	4,773	**4,825**	+1.1%	-5.1%
Birds						
Domestic fowl	341,763	390,340	423,737	**413,885**	-2.3%	+21.1%
Turkey	0	0	0	**17,234**	NA	NA
Other birds	94,553	107,878	86,371	**69,359**	-19.7%	-26.6%
Reptiles						
Reptiles	1,544	1,972	2,072	**2,433**	+17.4%	+57.6%
Amphibians						
Rana	3,563	6,169	1,722	**2,792**	+62.1%	-21.6%
Xenopus	14,074	17,386	17,806	**20,523**	+15.3%	+45.8%
Other amphibians	6,775	19,944	18,293	**16,572**	-9.4%	+144.6%
Fish						
Zebra fish	259,468	318,426	277,328	**340,316**	+22.7%	+31.2%
Sea bass	0	0	0	**646,076**	NA	NA
Salmon, trout, chars and graylings	0	0	0	**1,914,360**	NA	NA
Guppy, swordtail, molly, platy	0	0	0	**88,787**	NA	NA
Other fish	2,211,383	1,949,351	1,914,039	**440,091**	-77%	-80.1%
Cephalopods						
Cephalopods	4,268	16,968	1,633	**3,084**	+88.9%	-27.7%
Totals						
Total	**8,822,404**	**8,579,439**	**7,938,064**	**9,406,233**	**+18.5%**	**+6.6%**

Table 5: Numbers of animals used for the first time by types of species between 2018 and 2021

The number of mammals used for the first time in 2021 increased (+4.5%) compared to 2020 but remained below those reported in 2018 (-7.7%). This could be interpreted by a lower use of mammals in 2020 due to the COVID-19 pandemics that impacted basic and applied research where mammals are mostly used while the longer trend shows a tendency to reduce the use of mammalian species.

Looking at the evolution of species used since 2018, the following tend to be more often used the first time: hamsters (Syrian) (+59.4% vs 2020, +194.4% vs 2018), zebra fish (+22.7% vs 2020, +31.2% vs 2018), xenopus (+15.3% vs 2020, +45.8% vs 2018), rhesus monkeys (+22.5% vs 2020, +32.5% vs 2018) and rabbits (+3.7% vs 2020, +7.9% vs 2018). On the contrary, guinea pigs (-2.1% vs 2020, -11.9% vs 2018) and rats (+2.6% vs 2020 but -17.7% vs 2018) tend to be less often used.

In 2021, non-human primate first uses increased slightly compared to 2020 (+11.1%) but still decreased compared to 2018 (-13%). First uses of the following decreased versus 2020: vervets (Chlorocebus spp.) (-91.2%), baboons (-17%), mammosets and tamarins (-13.3%). Nevertheless, none of them show a real decreasing trend when looking at years 2019 and 2018.

Last, sea bass (646,076), salmon, trouts, chars and graylings (1,914,360), guppy, swordtail, molly, platy (88,787), turkey (17,234) had no reported first uses in 2020 as these species categories were introduced for the first time in 2021. This led to a decrease of "Other fish" first uses by -77% and "Other birds" by -19.7% as intended.

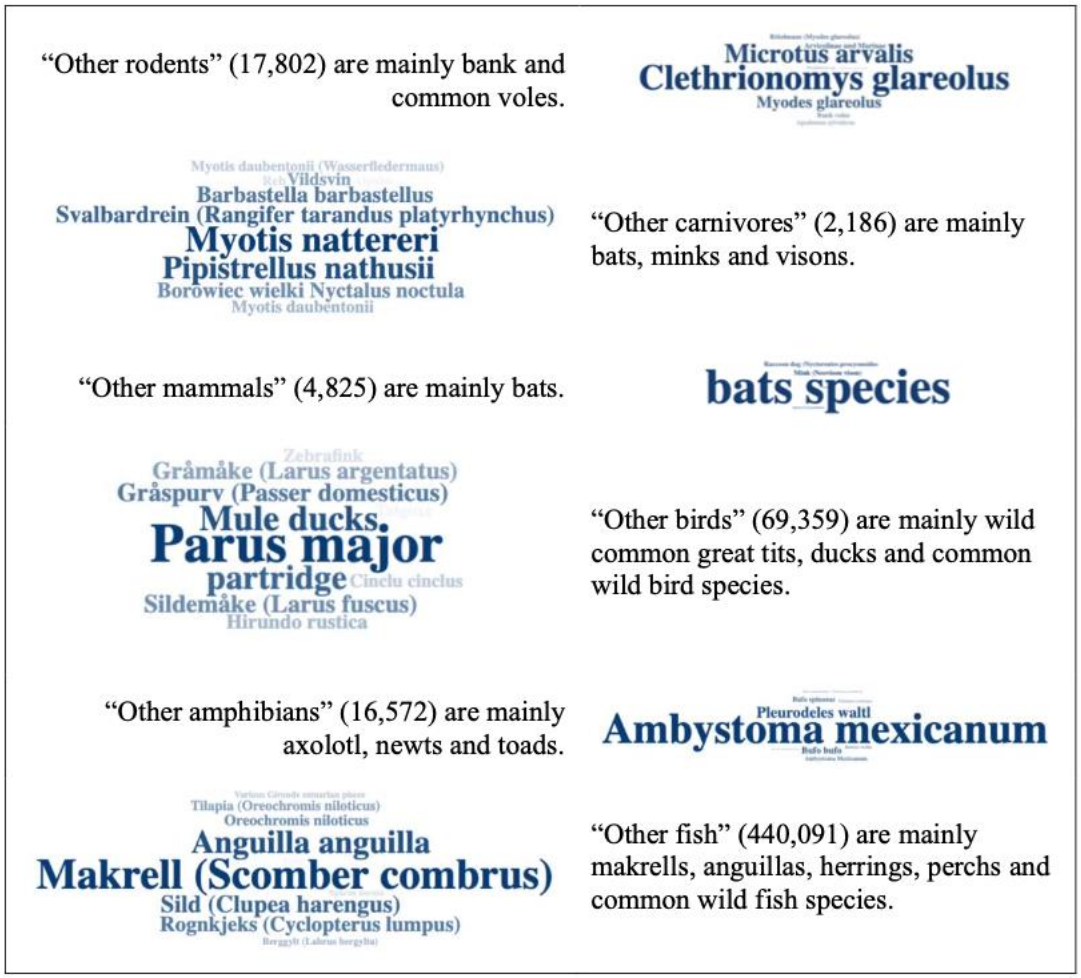


Figure 6: Examples of "Other species" used for the first time in 2021

Looking at the Member States narratives, figure 6 shows some examples of the main species reported under "Other" species categories. "Other carnivores" (-68.2%), "Other rodents" (-36.8%), "Other amphibians" (-9.4%) decreased as well compared to 2020 (Table 5).

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)

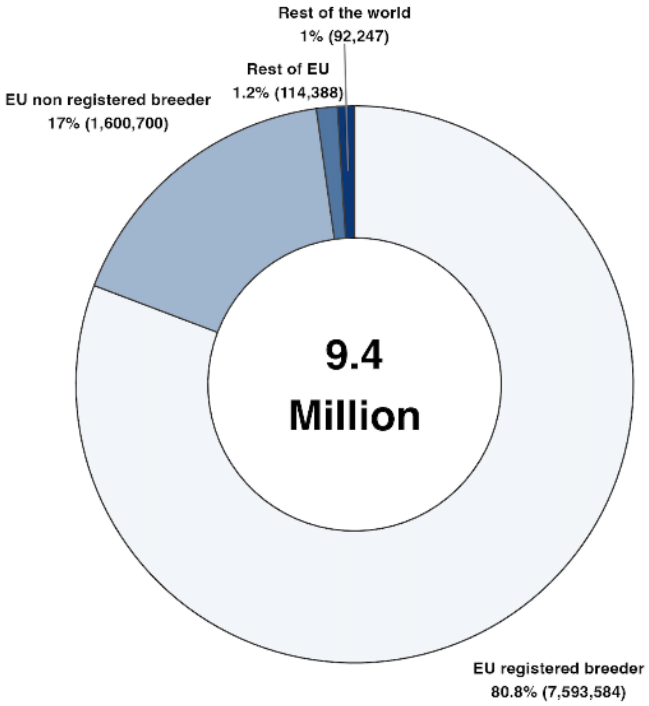


Figure 7: Place of birth of animals other than non-human primates in 2021

In 2021, 80.8% of the animals used for scientific purposes for the first time were born in the Union at registered breeders[15] and 2.2% were born outside of the Union (either in the rest of Europe or outside of Europe). Categories of animals born in the Union but not at a registered breeder includes animals from, for example, farms, and studies carried out using wild animals, especially wild fish (Figure 7).

In 2021, the proportion of animals born in the EU but not at a registered breeder increased to 17% (+2.7 points) while the proportion of animals born in the EU at a registered breeder decreased (-3.3 points) (Table 6).

	2018	2019	2020	2021
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)**
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)**
Animals born in rest of Europe	2.4% (208,244)	0.8% (67,351)	0.6% (50,783)	**1.2% (114,388)**

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

	2018	2019	2020	2021
Animals born in rest of world	0.9% (79,712)	0.7% (62,262)	1% (75,961)	**1% (92,247)**
Total	**100% (8,816,293)**	**100% (8,574,120)**	**100% (7,933,280)**	**100% (9,400,919)**

Table 6: Place of birth of animals other than non-human primates between 2018 and 2021

The increase of species most bred in the EU but not at a registered breeder (Table 7) is mainly due to the increase of fish used for the first time as they mostly come from this origin: 222,752 other fish (50.6%), 827,856 salmon, trout, chars and graylings (43.2%) and 119,983 sea bass (18.5%). These particular species are those used by the three projects responsible for the important increase of fish used for the first time in 2021.

Other species commonly coming from non-registered breeders in the EU are rana (94.3%), reptiles (81.3%) and farm animals such as cattle (66.6%), horse, donkeys and cross-breeds (65%), pigs (60.9%), sheep (58.6%) or goats (47%).

Dogs (41.3%) and cats (55.9%) are also often coming from the EU but not at 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
Rana	**94.3%**	2,632
Reptiles	**81.3%**	1,978
Other mammals	**78%**	3,762
Cattle	**66.6%**	14,702
Horses, donkeys and cross-breeds	**65%**	2,023
Other birds	**61.2%**	42,442
Pigs	**60.9%**	47,589
Other rodents	**60.6%**	10,789
Sheep	**58.6%**	9,173
Cats	**55.9%**	1,129
Other fish	**50.6%**	222,752
Goats	**47%**	510
Salmon, trout, chars and graylings	**43.2%**	827,856
Dogs	**41.3%**	4,185
Other amphibians	**38.7%**	6,413
Cephalopods	**35.5%**	1,096
Other carnivores	**28.6%**	626
Domestic fowl	**28.3%**	116,983
Sea bass	**18.5%**	119,410
Turkey	**13%**	2,243

Table 7: Main species bred in in EU but not at a registered breeder 2021

Species coming from the rest of the Europe (Table 8) are other fish (13.5%), birds (13.1%) or mammals (9.2%), reptiles (9.5%) or xenopus (9.1%). These include animals coming from the wild used for preservation of species or protection of the natural environment.

	Percent	Number
Other fish	**13.5%**	59,549
Other birds	**13.1%**	9,073
Reptiles	**9.5%**	231

	Percent	Number
Other mammals	**9.2%**	446
Xenopus	**9.1%**	1,875

Table 8: Main species bred in the rest of Europe in 2021

Last, other carnivores (67.9%) and to a lesser extent dogs (20.3%) and cats (10.3%) are more likely to come from the rest of the world (Table 9).

	Percent	Number
Other carnivores	**67.9%**	1,485
Dogs	**20.3%**	2,058
Cats	**10.3%**	209

Table 9: Main species bred in the rest of the world in 2021

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 2021, the three main sources of non-human primates were Africa, Asia and EU-registered breeders represented 99.5% of non-human primates used for scientific purposes (Figure 8).

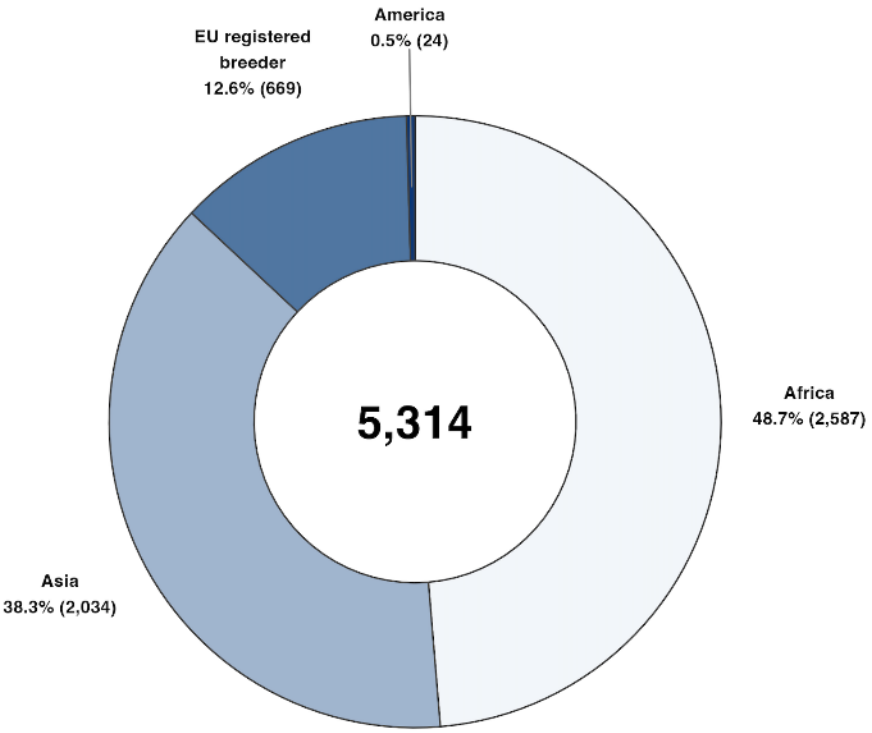


Figure 8: Source of non-human primates in 2021

Since 2018, the proportion of non-human primates born at an EU registered breeder remain stable being set at 12.6% in 2021. The number of animals born in Asia decreased slightly compared to 2018 and 2019 to 38.3% while the number of animals born in Africa increased to 48.7%. 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. No clear trend emerges since 2018 with regard to the origin of non-human primates (Table 10).

	2018	2019	2020	2021	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%)**	+4%	-20.5%
Animals born in rest of Europe	0 (0%)	0 (0%)	1 (0%)	**0 (0%)**	-100%	NA
Animals born in Asia	2,737 (44.8%)	2,418 (45.5%)	1,584 (33.1%)	**2,034 (38.3%)**	+28.4%	-25.7%
Animals born in America	12 (0.2%)	18 (0.3%)	36 (0.8%)	**24 (0.5%)**	-33.3%	+100%
Animals born in Africa	2,466 (40.4%)	1,917 (36%)	2,291 (47.9%)	**2,587 (48.7%)**	+12.9%	+4.9%
Animals born elsewhere	54 (0.9%)	282 (5.3%)	229 (4.8%)	**0 (0%)**	-100%	-100%
Total	**6,111 (100%)**	**5,319 (100%)**	**4,784 (100%)**	**5,314 (100%)**	**+11.1%**	**-13%**

Table 10: Evolution of non-human primates source between 2018 and 2021

In 2021, cynomolgus monkeys still represented the majority of non-human primates used for the first time (89.3%) 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 Asia	Animals born in America	Animals born in Africa
Prosimians	59 (100%)	0 (0%)	0 (0%)	0 (0%)
Marmoset and tamarins	170 (100%)	0 (0%)	0 (0%)	0 (0%)
Cynomolgus monkey	140 (2.9%)	2,020 (42.6%)	0 (0%)	2,587 (54.5%)
Rhesus monkey	243 (87.4%)	14 (5%)	21 (7.6%)	0 (0%)
Vervets (Chlorocebus spp.)	0 (0%)	0 (0%)	3 (100%)	0 (0%)
Baboons	44 (100%)	0 (0%)	0 (0%)	0 (0%)
Squirrel monkey	13 (100%)	0 (0%)	0 (0%)	0 (0%)
Total	**669 (12.6%)**	**2,034 (38.3%)**	**24 (0.5%)**	**2,587 (48.7%)**

Table 11: Source of non-human primates by species in 2021

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

From 2021, the reporting of whether a non-human primate is sourced from a self-sustaining colony is separated from the reporting of its generation. In 2021, of all the non-human primate species bred in captivity, the majority came from self-sustaining colonies (55.8%). Cynomolgus monkeys (47.5%) and marmoset and tamarins (34.1%) are more often coming from non-self-sustaining colonies than prosimians, vervets (Chlorocebus spp.) and baboons that are all coming from self-sustaining colonies (Table 12).

	Self-sustaining colony	Non self-sustaining colony
Prosimians	59 (100%)	0 (0%)
Marmoset and tamarins	112 (65.9%)	58 (34.1%)
Cynomolgus monkey	2,493 (52.5%)	2,254 (47.5%)
Rhesus monkey	242 (87.1%)	36 (12.9%)
Vervets (Chlorocebus spp.)	3 (100%)	0 (0%)
Baboons	44 (100%)	0 (0%)
Squirrel monkey	10 (76.9%)	3 (23.1%)
Total	**2,963 (55.8%)**	**2,351 (44.2%)**

Table 12: Non-human primates colony type by species in 2021

With regard to the source of non-human primates, animals born in Africa are more often coming from non self-sustaining colonies (65.4%) (Table 13).

	Self-sustaining colony	Non self-sustaining colony
Animals born at a registered breeder within EU	564 (84.3%)	105 (15.7%)
Animals born in Asia	1,481 (72.8%)	553 (27.2%)
Animals born in America	24 (100%)	0 (0%)
Animals born in Africa	894 (34.6%)	1,693 (65.4%)
Total	**2,963 (55.8%)**	**2,351 (44.2%)**

Table 13: Non-human primates colony type by source in 2021

Most of non-human primates are from the second generation or higher (82.4%). 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 34% in 2021 (Table 14). No animal coming from the wild was reported in 2021 (F0).

	F2 or greater	F1
Animals born at a registered breeder within EU	626 (93.6%)	43 (6.4%)
Animals born in Asia	2,020 (99.3%)	14 (0.7%)
Animals born in America	23 (95.8%)	1 (4.2%)
Animals born in Africa	1,708 (66%)	879 (34%)
Total	**4,377 (82.4%)**	**937 (17.6%)**

Table 14: Generation of non-human primates by source in 2021

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 2021, the total number of all uses (first use and any subsequent reuse) for the purposes of research, testing, routine production and education is 9,51 million. This is an increase of +18.1% compared to 2020 but limited to +5.9% compared to 2018 (Table 15).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Basic research	3,982,963 (44.4%)	3,668,904 (42.1%)	3,293,349 (40.9%)	**3,022,541 (31.8%)**	-8.2%	-24.1%
Translational and applied research	2,650,730 (29.5%)	2,535,951 (29.1%)	2,510,499 (31.2%)	**3,862,731 (40.6%)**	+53.9%	+45.7%
Regulatory use	1,622,816 (18.1%)	1,482,372 (17%)	1,404,240 (17.4%)	**1,317,252 (13.8%)**	-6.2%	-18.8%
Routine production	354,209 (3.9%)	438,236 (5%)	406,860 (5.1%)	**412,889 (4.3%)**	+1.5%	+16.6%
Other	368,363 (4.1%)	589,761 (6.8%)	439,982 (5.5%)	**897,801 (9.4%)**	+104.1%	+143.7%
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**+18.1%**	**+5.9%**

Table 15: Total number of uses of animals by main purpose categories between 2018 and 2021

IV.2.1.1. Main categories of scientific purposes

In 2021, most uses were conducted for research purposes (72.4%) with 40.6% of the uses being carried out for translational and applied research purposes and 31.8% for basic research. A further 13.8% of animal uses in procedures were carried out for regulatory use to satisfy regulatory requirements, while routine production concerned 4.3% of uses.

Other categories (9.4%) 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). Protection of species increased also significantly compared to 2020 (+452.1%) explained by Norway in the Member State narrative by a correction of previously wrong reporting in this category.

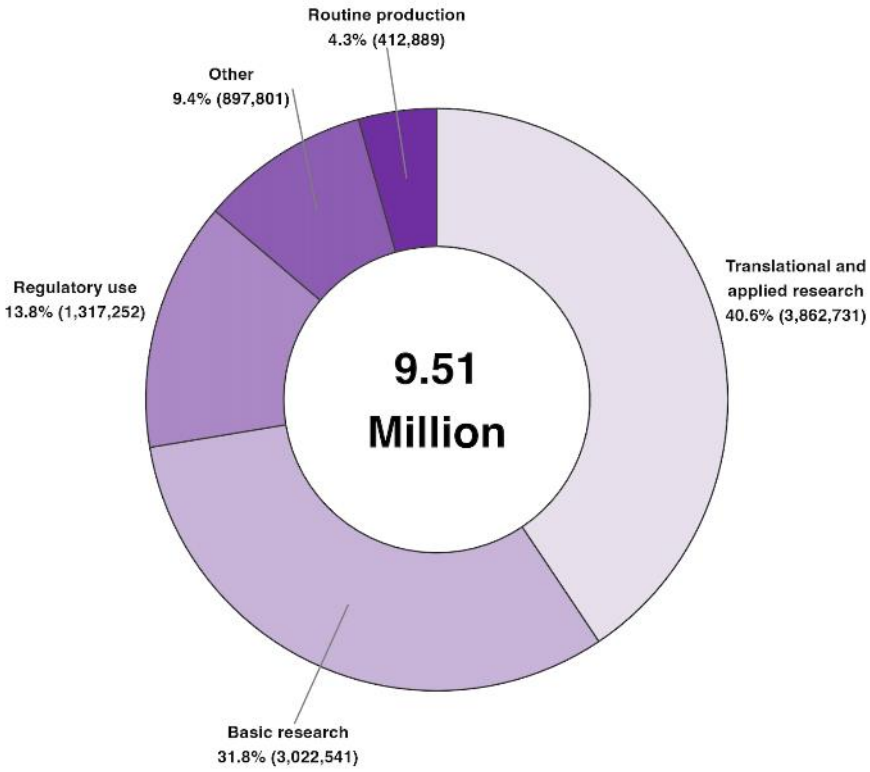


Figure 9: Uses of animals used for scientific purposes in 2021

Uses for translational and applied research increased the most (+53.9%) compared to 2020. This increase can be explained by the 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).

Basic research decreased (-8.2%), a change that can be explained by the introduction of new reporting categories, which resulted in a significant decrease of uses reported under "Other basic research". It would seem that categories developmental biology under basic research and animal nutrition under applied research have received the vast majority of entries previously reported under this category. Lastly, regulatory uses continue to decrease (-8.2%) with routine uses remained almost stable (+1.5%) (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 2021, 46.2% of uses, were reported as 'mild' (up to and including), 40.6% as 'moderate', and 9.7% as 'severe' while 3.5% of uses were reported as 'non-recovery'. Compared to 2020, the number of uses reported as non-recovery decreased proportionally in 2021 (-1.4%) while those having resulted in moderate severity increased (+3%) (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 or training.

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

Table 16: Severity of uses reported in 2021

In 2021, 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 (54.6%); regulatory testing for neurotoxicity testing (40.5%), acute toxicity testing (38.6%), ecotoxicity testing (31.7%) and batch potency testing for quality checks (25.2%); in basic research for musculoskeletal system (21.7%); in translational and applied research for human immune disorders (21.1%), human musculoskeletal disorders (20.8%), animal diseases and disorders (18.8%), human infectious disorders (16.5%) and diagnosis of diseases (16.4%).

In absolute numbers, the most important number of severe uses was reported for animal diseases and disorders (183,876), batch potency testing (134,502), human infectious disorders (32,227) and monoclonal antibody production by mouse ascites method (20,135).

On the contrary, the lowest number of severe uses for specific procedures were reported for forensic inquiries (0); for preservation of species (0); for training for the acquisition, maintenance or improvement of vocational skills (0.5%); for higher education (0.8%); in routine production for monoclonal and polyclonal antibodies production (excluding mouse ascites method) (0%) and blood based products (0.2%); in regulatory use for phototoxicity testing (0%), genotoxicity testing (0.1%), kinetics (0.6%), pyrogenicity testing for quality checks (0.7%); in basic research for urogenital/reproductive system (2.3%); and in translational and applied research for animal nutrition (0.9%) and animal welfare (1.1%) (Figure 10).

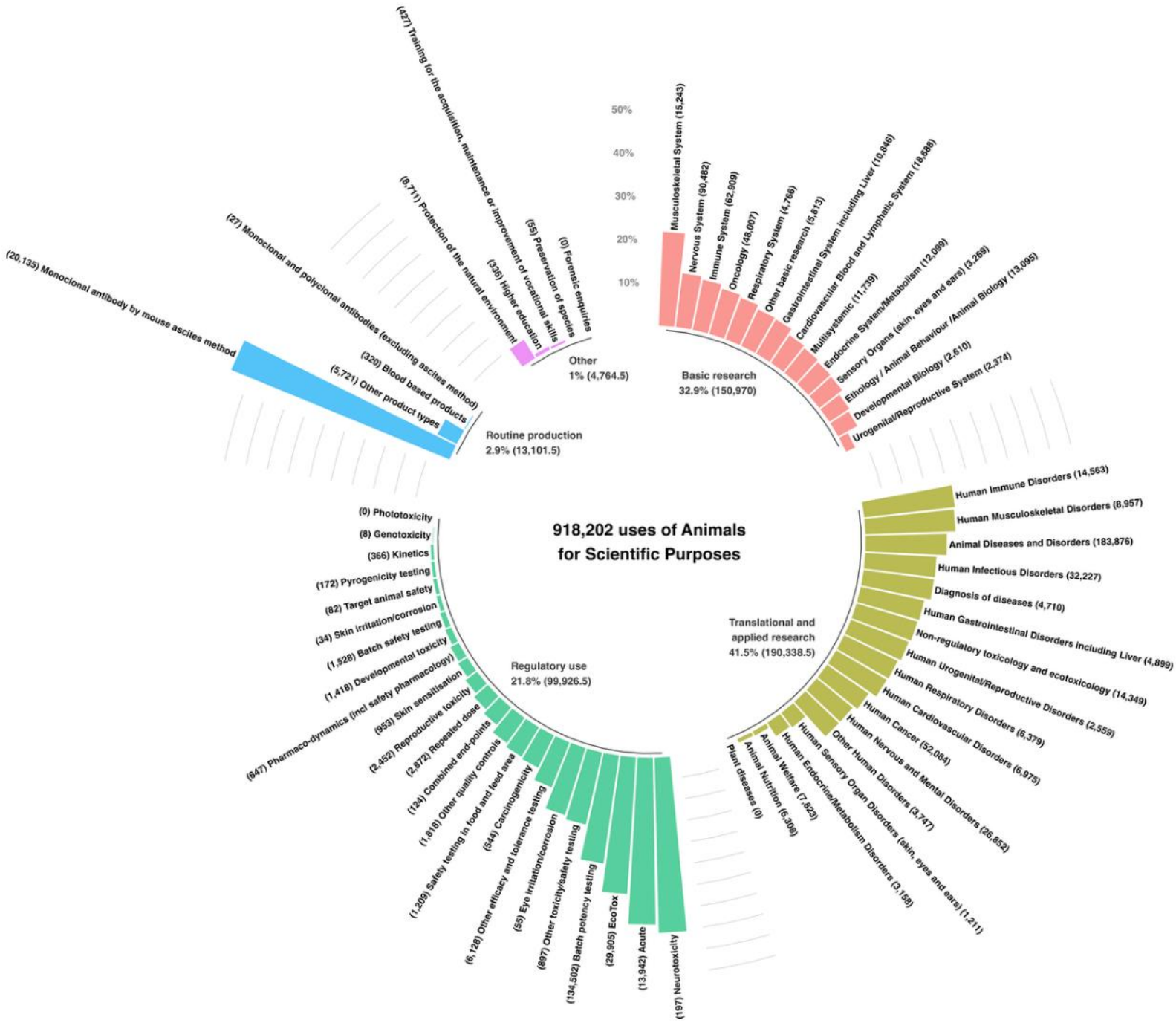


Figure 10: proportion of severe uses of animals by detailed purposes in 2021

IV.2.2. Detailed information on use purposes

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Basic research						
Oncology	432,191	446,116	414,164	**443,223**	+7%	+2.6%
Cardiovascular Blood and Lymphatic System	254,931	240,688	213,763	**241,922**	+13.2%	-5.1%
Nervous System	687,420	738,363	640,405	**722,137**	+12.8%	+5.1%
Respiratory System	52,932	50,869	43,240	**46,783**	+8.2%	-11.6%
Gastrointestinal System including Liver	141,132	131,664	122,515	**119,086**	-2.8%	-15.6%
Musculoskeletal System	76,753	89,109	66,665	**70,091**	+5.1%	-8.7%
Immune System	547,768	528,229	506,177	**534,775**	+5.6%	-2.4%
Urogenital/Reproductive System	83,859	84,852	84,777	**104,037**	+22.7%	+24.1%
Sensory Organs (skin, eyes and ears)	52,418	59,362	51,324	**49,117**	-4.3%	-6.3%
Endocrine System/Metabolism	187,421	180,441	152,863	**175,901**	+15.1%	-6.1%
Developmental Biology	0	0	0	**52,320**	NA	NA
Multisystemic	309,585	175,765	236,967	**160,247**	-32.4%	-48.2%
Ethology / Animal Behaviour /Animal Biology	988,510	724,065	637,819	**240,084**	-62.4%	-75.7%
Other basic research	168,043	219,381	122,670	**62,818**	-48.8%	-62.6%
Translational and applied research						
Human Cancer	450,538	439,884	416,399	**444,519**	+6.8%	-1.3%
Human Infectious Disorders	190,731	224,597	192,464	**195,281**	+1.5%	+2.4%
Human Cardiovascular Disorders	66,479	60,283	55,682	**51,854**	-6.9%	-22%
Human Nervous and Mental Disorders	259,148	257,900	209,497	**251,175**	+19.9%	-3.1%
Human Respiratory Disorders	54,579	46,101	46,248	**46,558**	+0.7%	-14.7%
Human Gastrointestinal Disorders including Liver	37,868	41,399	35,462	**32,676**	-7.9%	-13.7%
Human Musculoskeletal Disorders	38,092	33,562	38,250	**43,090**	+12.7%	+13.1%
Human Immune Disorders	85,686	68,609	69,893	**69,092**	-1.1%	-19.4%
Human Urogenital/Reproductive Disorders	11,850	17,535	14,318	**17,864**	+24.8%	+50.8%
Human Sensory Organ Disorders (skin, eyes and ears)	35,175	38,339	30,289	**28,989**	-4.3%	-17.6%
Human Endocrine/Metabolism Disorders	115,102	128,928	94,337	**85,898**	-8.9%	-25.4%
Other Human Disorders	26,505	22,744	21,316	**34,910**	+63.8%	+31.7%
Animal Diseases and Disorders	937,444	850,073	505,383	**976,663**	+93.3%	+4.2%
Animal Nutrition	0	0	0	**722,635**	NA	NA
Animal Welfare	137,426	205,137	688,564	**735,313**	+6.8%	+435.1%
Diagnosis of diseases	143,637	45,873	40,999	**28,660**	-30.1%	-80%
Plant diseases	38	108	106	**22**	-79.2%	-42.1%
Non-regulatory toxicology and ecotoxicology	60,432	54,879	51,292	**97,532**	+90.2%	+61.4%
Regulatory use						
Quality control (incl batch safety and potency testing)						
Batch safety testing	137,324	128,459	137,518	**129,667**	-5.7%	-5.6%
Pyrogenicity testing	29,815	30,687	24,139	**23,695**	-1.8%	-20.5%
Batch potency testing	752,958	643,369	577,534	**533,527**	-7.6%	-29.1%
Other quality controls	22,030	22,048	20,541	**25,516**	+24.2%	+15.8%
Toxicity and other safety testing including pharmacology						
Acute and sub-acute toxicity testing methods						
LD50, LC50	23,765	21,044	16,421	**16,992**	+3.5%	-28.5%
Other lethal methods	499	562	5,259	**4,025**	-23.5%	+706.6%
Non lethal methods	21,947	20,702	14,952	**15,100**	+1%	-31.2%
Skin irritation/corrosion	3,998	2,945	4,070	**3,824**	-6%	-4.4%
Skin sensitisation	37,606	38,854	38,024	**37,736**	-0.8%	+0.3%
Eye irritation/corrosion	840	452	491	**339**	-31%	-59.6%
Repeated dose toxicity						
up to 28 days	43,484	37,190	37,777	**36,775**	-2.7%	-15.4%
29 - 90 days	22,938	23,141	22,503	**21,720**	-3.5%	-5.3%
> 90 days	12,888	8,827	7,174	**9,159**	+27.7%	-28.9%
Carcinogenicity	3,233	2,650	1,569	**6,573**	+318.9%	+103.3%
Genotoxicity	4,342	4,094	4,808	**6,388**	+32.9%	+47.1%

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Reproductive toxicity	58,562	55,794	61,970	**75,041**	+21.1%	+28.1%
Developmental toxicity	63,271	68,330	68,022	**94,523**	+39%	+49.4%
Neurotoxicity	4,273	629	2,130	**486**	-77.2%	-88.6%
Kinetics	62,325	55,210	44,377	**62,180**	+40.1%	-0.2%
Pharmaco-dynamics (incl safety pharmacology)	78,934	72,909	56,047	**31,435**	-43.9%	-60.2%
Phototoxicity	519	414	114	**525**	+360.5%	+1.2%
Ecotoxicity						
Acute toxicity	54,115	51,523	51,860	**43,916**	-15.3%	-18.8%
Chronic toxicity	26,824	28,354	24,910	**31,499**	+26.5%	+17.4%
Reproductive ecotoxicity	240	224	2,653	**735**	-72.3%	+206.2%
Endocrine activity	790	8,784	13,679	**11,457**	-16.2%	+1350.3%
Bioaccumulation	3,511	3,733	1,977	**3,524**	+78.2%	+0.4%
Other ecotoxicity	3,339	3,706	2,034	**3,204**	+57.5%	-4%
Safety testing in food and feed area	41,208	37,600	31,124	**15,295**	-50.9%	-62.9%
Target animal safety	6,290	4,979	27,686	**10,225**	-63.1%	+62.6%
Combined end-points	0	0	0	**2,401**	NA	NA
Other toxicity/safety testing	4,145	16,922	14,305	**5,236**	-63.4%	+26.3%
Other efficacy and tolerance testing						
Other efficacy and tolerance testing	96,803	88,237	88,572	**54,534**	-38.4%	-43.7%
Routine production						
Blood based products	211,522	229,445	228,762	**155,994**	-31.8%	-26.3%
Monoclonal antibody by mouse ascites method	54,941	37,473	41,916	**36,882**	-12%	-32.9%
Monoclonal and polyclonal antibodies (excluding ascites method)	0	0	0	**105,580**	NA	NA
Other product types	87,746	171,318	136,182	**114,433**	-16%	+30.4%
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**	-22.7%	+45.4%
Preservation of species	83,683	224,921	106,058	**585,531**	+452.1%	+599.7%
Higher education	0	0	0	**43,879**	NA	NA
Training for the acquisition, maintenance or improvement of vocational skills	0	0	0	**93,945**	NA	NA
Higher education or training for the acquisition, maintenance or improvement of vocational skills	165,110	160,544	109,334	**0**	-100%	-100%
Forensic enquiries	273	357	105	**982**	+835.2%	+259.7%
Total	**8,979,081**	**8,715,224**	**8,054,930**	**9,513,214**	**+18.1%**	**+5.9%**

Table 17: Total number of uses of animals by detailed purpose categories between 2018 and 2021

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 increased by nearly 1.08 million between 2020 and 2021 (1.35 million increase in translational and applied research and 270,000 decrease in basic research).

Looking at the repartition of uses of animals for research and testing in 2021 (Table 17), the three most important categories of uses in 2021 are animal diseases and disorders, animal welfare and animal nutrition, which was not the case in previous reports. This is explained by the three projects using a very high number of fish in Spain and Norway in 2021 and by the fact the most of the "Other basic research" uses are now reported for animal nutrition that falls under the translational and applied research category.

Classifying the research purposes by research objectives centred on human, animal or other research[16], research centred on animals is subject to important variations between 2018 and 2021 while research focusing on human remains stable (despite a slight decrease in 2020 due to the COVID-19 pandemic).

The increase of uses in research is mainly due to the increase of research centred on animals (+46%) and to a lesser extent for research targeting humans +6.6%) compared to 2020. Other research decreased mainly because of the "Other basic research" decrease (table 18).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Human centred research	4,341,800 (65.5%)	4,151,212 (66.9%)	3,798,014 (65.4%)	4,050,205 (58.8%)	+6.6%	-6.7%
Animal centred research	2,063,380 (31.1%)	1,779,275 (28.7%)	1,831,766 (31.6%)	2,674,695 (38.8%)	+46%	+29.6%
Other research	228,513 (3.4%)	274,368 (4.4%)	174,068 (3%)	160,372 (2.3%)	-7.9%	-29.8%
Total	**6,633,693 (100%)**	**6,204,855 (100%)**	**5,803,848 (100%)**	**6,885,272 (100%)**	**+18.6%**	**+3.8%**

Table 18: Total number of uses for research purposes between 2018 and 2021

Comparing the main species used in research centred on humans, mice (82.2%), fish (zebra fish in particular) (7.4%) and rats (7.3%), with the main species use in research focusing on animals, which are fish (82%), birds (11.3%) and other mammals (farm animals in particular) (3.9%), shows that the species used for these two purposes are extremely different (Figure 11).

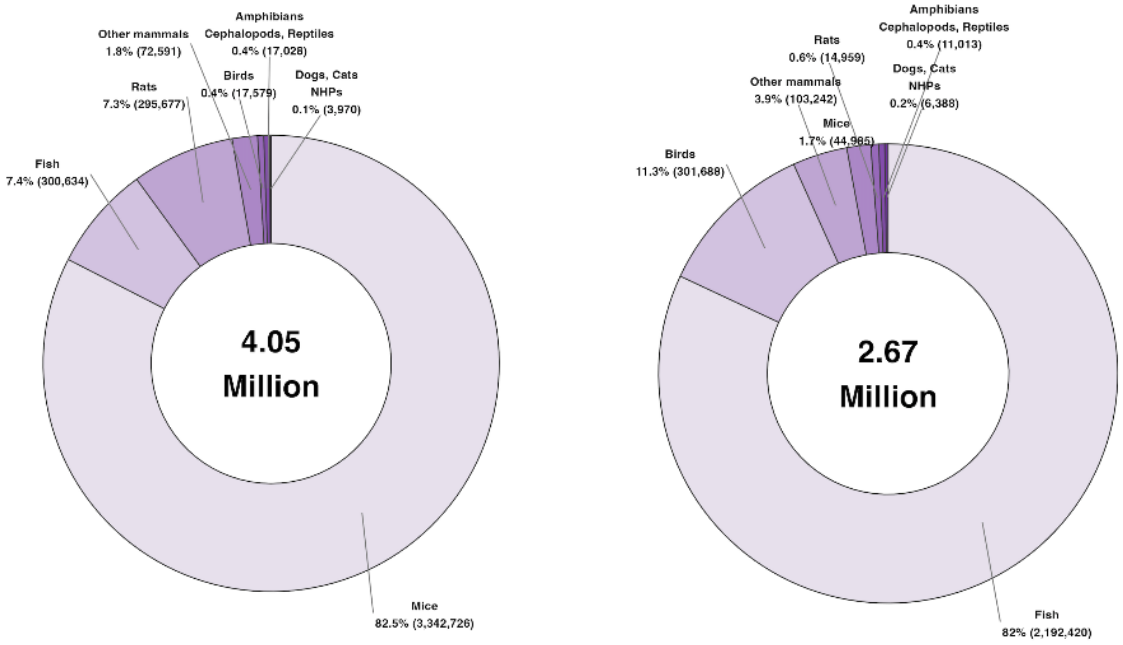


Figure 11: Main species used for human research (left) and animal research (right) in 2021

[16] 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.

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 importantly between years.

IV.2.2.1.1 Basic research

Basic research was the second main area for which animals were used, with more than three million uses in 2021. This is a decrease compared to 2018-2020 but this is also due to the creation of the animal nutrition category that triggered a move of uses reported under ethology/animal behaviour/animal biology to this new category under translational and applied research (from main category basic research).

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).

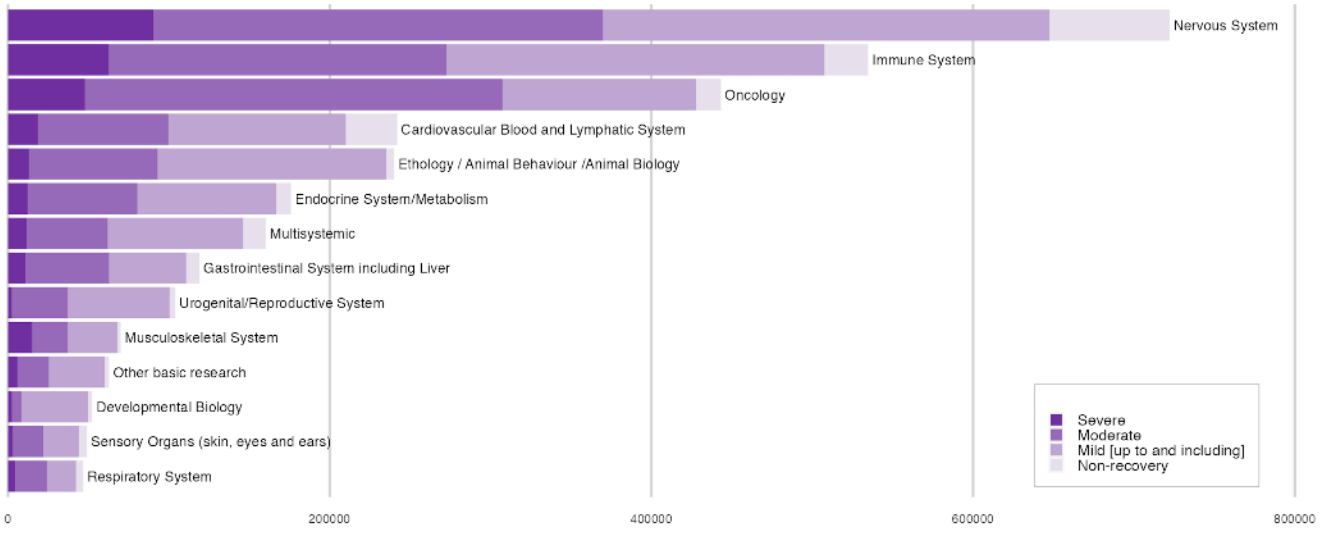


Figure 12: Basic research related uses by type of research and severity in 2021

In 2021, the decreases in the two main categories compared to 2020, ethology/animal behaviour/animal biology (-62.4%) and other basic research (-48.8%) are due to the change of reporting introduced by the directive update. Indeed, most of ethology/animal behaviour/animal biology uses and now reported under animal nutrition while other basic research uses related to developmental biology are now reported in a newly created category. For other categories of uses, uses in category urogenital/reproductive system increased (+22.7%) while multisystemic decreased (-32.4%) compared to 2020 (Table 17).

“Other basic research” included, for example, studies on evolutionary genetics, parasitology or embryo transfer. Comparing to 2018-2020 numbers, it is not possible to identify clear trends in basic research.

IV.2.2.1.2. Translational and applied research

Translational and applied research accounted for more than 3.8 million uses of animals in 2021, an important increase compared to 2020 (+53.9%). The three main areas of translational and applied research were animal diseases and disorders, animal welfare and animal nutrition that together account for more than 65% of all uses in translational and applied research (Figure 13).

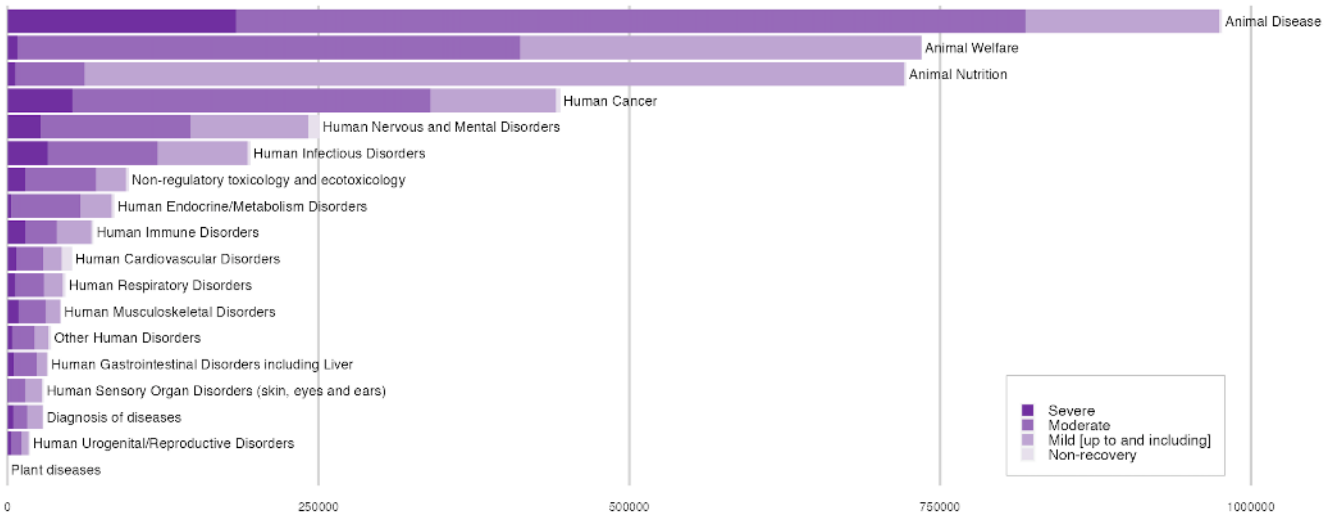


Figure 13: Translational and applied research related uses by type of research and severity in 2021

The categories that increased in 2021 compared to 2020 are animal diseases and disorders (+93.3%), non-regulatory toxicology and ecotoxicology (+90.2%), other human disorders (+68.3%), human urogenital/reproductive disorders (+22.7%) and human nervous and mental disorders (+19.9%). The newly introduced category animal nutrition reported 722,635 uses, the third highest number after animal welfare disease and disorders (976,663) and animal welfare (735,313).

The main categories that decreased in 2021 compared to 2020 average are plant diseases (-79.2% but for a very limited number of uses: 22), diagnosis of diseases (-30.1%), human endocrine/metabolism disorders (-8.9%) and human cardiovascular disorders (-6.9%).

Looking at longer term trends, diagnosis of diseases (-80%), human cardiovascular (-22%) disorders decreased continuously since 2018. On the contrary, animal welfare is continuously increasing (+435.1% compared to 2018) (table 17).

“Other human disorders” includes areas such as immuno-oncology, 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 2021, regulatory uses accounted for 1.32 million uses. The total number of uses for regulatory purposes decreased by -6.2% compared to 2020 and -18.8% compared to 2018. 54.1% of these uses were related to quality control (including batch safety and potency testing), 41.8% related to toxicity and other safety testing including pharmacology and the remainder (4.1%) were for other efficacy and tolerance testing. These three categories all continuously decreased since 2018 (table 19).

	2018	2019	2020	2021	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%)**	-6.2%	-24.4%
Toxicity and other safety testing including pharmacology	583,886 (36%)	569,572 (38.4%)	555,936 (39.6%)	**550,313 (41.8%)**	-1%	-5.7%
Other efficacy and tolerance testing	96,803 (6%)	88,237 (6%)	88,572 (6.3%)	**54,534 (4.1%)**	-38.4%	-43.7%
Total	**1,622,816 (100%)**	**1,482,372 (100%)**	**1,404,240 (100%)**	**1,317,252 (100%)**	**-6.2%**	**-18.8%**

Table 19: Regulatory uses by type of research between 2018 and 2021

The main species used for regulatory purposes are mice (37.9%), rats (25%), “Other mammals” (14.4%), birds (10.8%) and fish (10.5%) (Figure 14).

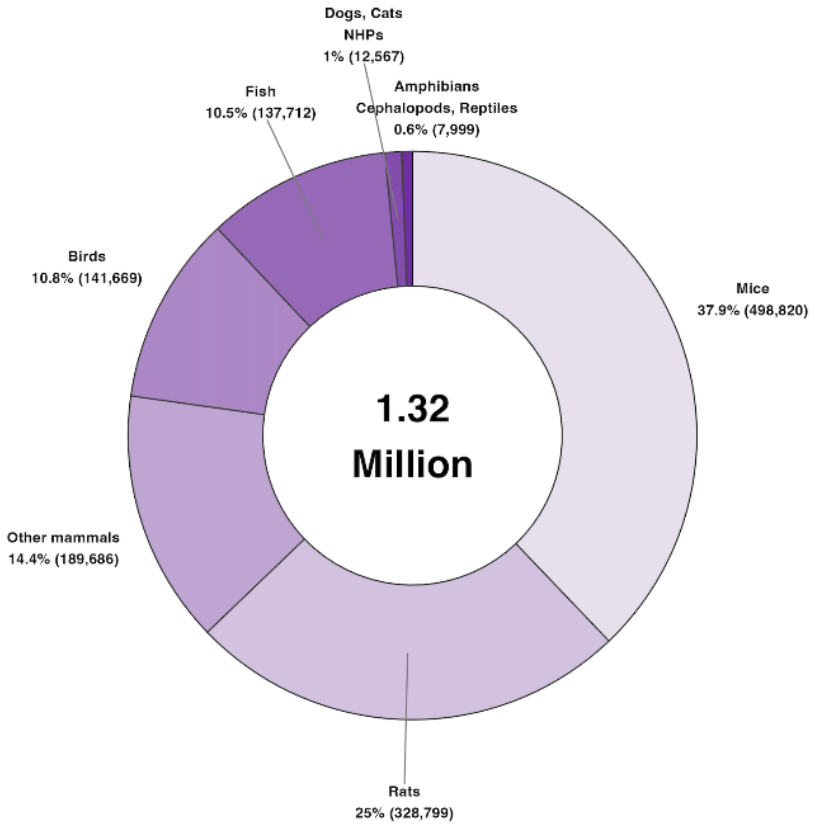


Figure 14: Main species used for regulatory purposes in 2021

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 712,405 uses in 2021. A large majority of these uses were related to batch potency testing purposes (74.9%), a severe procedure (134,502 uses in 2021) (figure 16). Nevertheless, this specific category is continuously decreasing (-29.1% compared to 2018) a trend that started since 2015 (see previous reports).

“Other quality controls” increased in 2021 compared to 2020 (+24.2%) and are related for example to pathogenicity tests, product purity or potency.

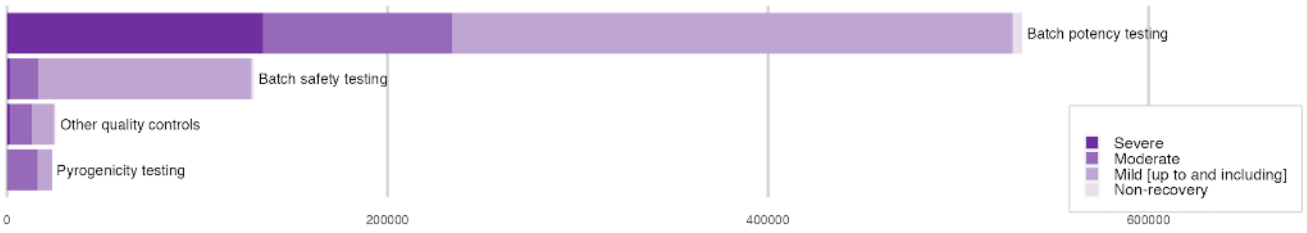


Figure 15: Quality control related uses by type of use and severity in 2021

In 2021, pyrogenicity testing, for which alternative methods (applicable to most use cases) are available, decreased compared to 2018 (-20.5%), continuing the downward trend despite of the temporary increase in 2019. In 2021, these uses are reported mainly by three countries covering 83% of all pyrogenicity testing, namely France (41%), Spain (29.8%), Bulgaria (13.1%). While Austria (-100%), Romania (-93.3%), Germany (-78.2%) and Italy (-31.7%) decrease significantly the uses of animal for pyrogenicity testing compared to 2018, Bulgaria saw a very important increase in 2021 (+2248.5%) compared to 2020 (Table 20). Member State specific data reveals this to be exclusively for the purposes of pyrogenicity testing.

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Austria	1,923 (6.4%)	0 (0%)	0 (0%)	**0 (0%)**	NA	-100%
Bulgaria	132 (0.4%)	300 (1%)	309 (1.3%)	**3,100 (13.1%)**	+903.2%	+2248.5%
Czech Republic	53 (0.2%)	51 (0.2%)	41 (0.2%)	**0 (0%)**	-100%	-100%
France	9,578 (32.1%)	12,007 (39.1%)	10,325 (42.8%)	**9,711 (41%)**	-5.9%	+1.4%
Germany	6,291 (21.1%)	6,457 (21%)	3,223 (13.4%)	**1,371 (5.8%)**	-57.5%	-78.2%
Hungary	20 (0.1%)	37 (0.1%)	14 (0.1%)	**0 (0%)**	-100%	-100%
Ireland	145 (0.5%)	525 (1.7%)	943 (3.9%)	**657 (2.8%)**	-30.3%	+353.1%
Italy	2,243 (7.5%)	2,177 (7.1%)	2,025 (8.4%)	**1,531 (6.5%)**	-24.4%	-31.7%

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Poland	192 (0.6%)	225 (0.7%)	186 (0.8%)	**177 (0.7%)**	-4.8%	-7.8%
Romania	372 (1.2%)	30 (0.1%)	0 (0%)	**25 (0.1%)**	NA	-93.3%
Slovenia	64 (0.2%)	51 (0.2%)	68 (0.3%)	**68 (0.3%)**	0%	+6.2%
Spain	8,802 (29.5%)	8,827 (28.8%)	7,005 (29%)	**7,055 (29.8%)**	+0.7%	-19.8%
Total	**29,815 (100%)**	**30,687 (100%)**	**24,139 (100%)**	**23,695 (100%)**	**-1.8%**	**-20.5%**

Table 20: Number of uses for pyrogenicity testing per country between 2018 and 2021

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 550,313 uses of animals in 2021, which corresponds to 5.8% of all uses of animals. Most of the uses in this area were related to developmental toxicity, reproductive toxicity, kinetics, acute toxicity for ecotoxicity testing (Figure 16).

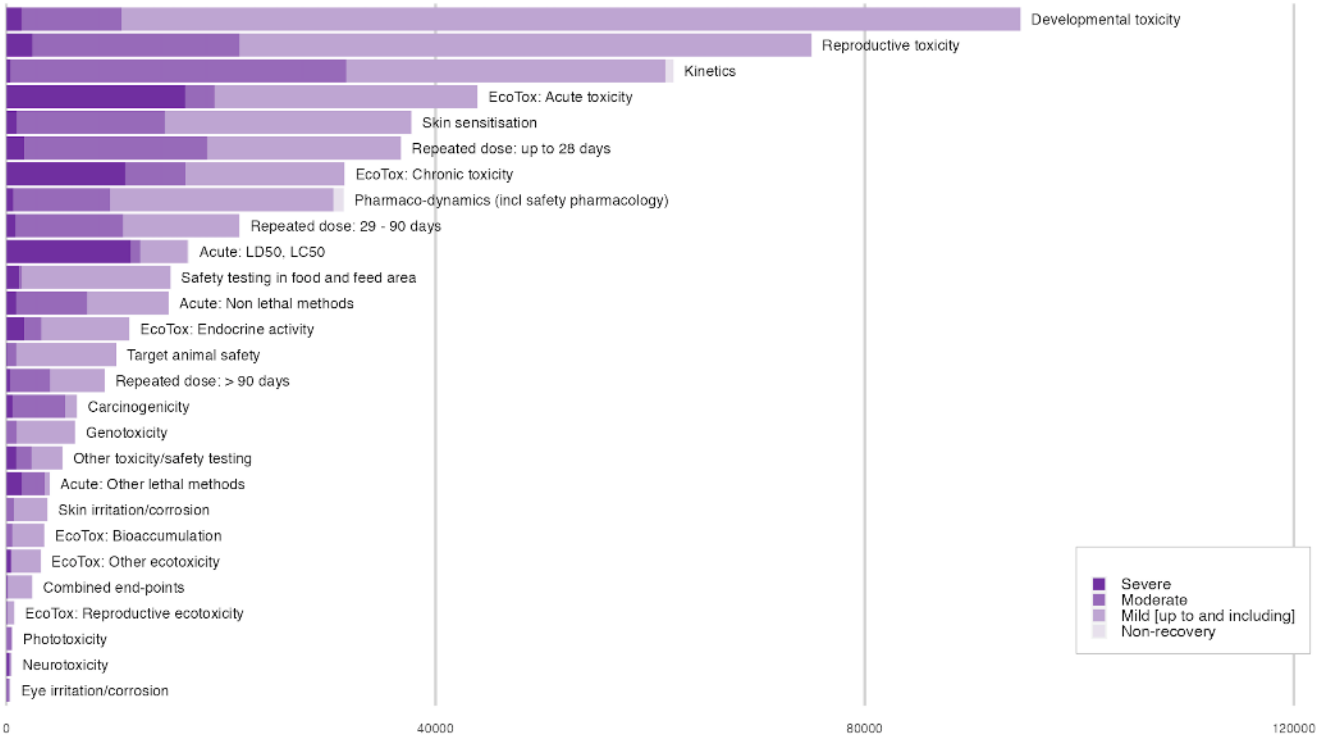


Figure 16: Toxicity and other safety testing including pharmacology by type of use and severity in 2021

Compared to the 2020, the total number of uses for toxicity and other safety testing including pharmacology decreased (-1%), which is a continuous trend since 2018 (-5.7%).

Carcinogenicity (+318.8%%), phototoxicity (+360.5%), bioaccumulation (+78.2%), "Other ecotoxicity" (57.5%), kinetics (+40.1%) and genotoxicity (32.9%) increased the most in 2021 compared to 2020. This does not represent a long-term trend, but a sudden increase in 2021.

A decrease in the number of uses was observed in 2021 compared to 2018 for the following areas: neurotoxicity (-77.2%), reproductive ecotoxicity (-72.3%), other toxicity/safety testing (-63.4%), target animal safety (-63.1%) without any long-term trend identified.

Some of these variations could be related to testing required by EU or national authorities creating temporary increase for specific endpoints during a year or two.

Positive signs of long-term decrease can nevertheless be observed for safety testing in the food and feed area (-50.9% vs 2020, -62.9% vs 2018), pharmaco-dynamics (incl safety pharmacology) (-43.9% vs 2020, -60.2%) and eye irritation/corrosion (-31% vs 2020, -59.6%) (Table 17).

"Other toxicity/safety testing" are related for example to the identification of residual pathogens in vaccines.

IV.2.2.2.2. Legislative aspects of regulatory uses

In 2021, 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 (51.1%), veterinary medicinal products (22.9%) and industrial chemicals (12.5%) (table 21).

	2018	2019	2020	2021	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%)**	-11.3%	-34.9%
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%)**	-5.5%	+13.6%
Medical devices legislation	52,335 (3.2%)	50,849 (3.4%)	50,427 (3.6%)	**48,932 (3.7%)**	-3%	-6.5%
Industrial chemicals legislation	123,837 (7.6%)	117,867 (8%)	122,736 (8.7%)	**165,086 (12.5%)**	+34.5%	+33.3%
Plant protection product legislation	46,189 (2.8%)	51,778 (3.5%)	67,174 (4.8%)	**55,798 (4.2%)**	-16.9%	+20.8%
Biocides legislation	1,788 (0.1%)	1,985 (0.1%)	4,442 (0.3%)	**2,578 (0.2%)**	-42%	+44.2%
Food legislation including food contact material	41,641 (2.6%)	34,252 (2.3%)	28,326 (2%)	**16,079 (1.2%)**	-43.2%	-61.4%
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%)**	+33.1%	+91.4%
Other legislation	49,445 (3%)	44,290 (3%)	42,024 (3%)	**39,465 (3%)**	-6.1%	-20.2%
Total	**1,622,816 (100%)**	**1,482,372 (100%)**	**1,404,240 (100%)**	**1,317,252 (100%)**	**-6.2%**	**-18.8%**

Table 21: Regulatory uses by type of legislation between 2018 and 2021

Between 2018 and 2021, regulatory uses saw a constant decrease (-18.8%) mainly due to the decrease of uses for legislation on medicinal products for human use (-34.9%). In the same period, food legislation including food contact material (-61.4%) and "Other legislation" uses decreased as well (-20.2%) (table 21).

Food legislation including food contact material included still in 2018, for example, the use of mouse bioassay for the purposes of shellfish toxin testing. As provided by Regulation 2017/1980[17], the mouse bioassay could still be used as reference method until the end of 2018, after which the alternative, the so-called Lawrence method, should have replaced its use.

In 2021, uses for feed legislation including legislation for the safety of target animals, workers and environment (+33.1%) and industrial chemicals legislation (+34.5%) are the two legislations that saw an increase compared to 2020.

In 2021, the majority of regulatory uses were performed to satisfy regulatory requirements originating from the Union (96.2%). Non-EU requirements accounted for 2.5% and national requirements for 1.3% (Table 22)

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

Table 22: Regulatory uses by origin of regulatory requirement between 2018 and 2021

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, ICH18 and VICH19 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	452,614 (67.2%)	197,692 (29.4%)	23,103 (3.4%)
Legislation on medicinal products for veterinary use and their residues	256,192 (84.8%)	27,487 (9.1%)	18,446 (6.1%)
Medical devices legislation	2,399 (4.9%)	45,854 (93.7%)	679 (1.4%)
Industrial chemicals legislation	0 (0%)	165,086 (100%)	0 (0%)
Plant protection product legislation	0 (0%)	55,798 (100%)	0 (0%)
Biocides legislation	0 (0%)	2,213 (85.8%)	365 (14.2%)
Food legislation including food contact material	448 (2.8%)	15,631 (97.2%)	0 (0%)
Feed legislation including legislation for the safety of target animals, workers and environment	0 (0%)	2,300 (16.7%)	11,480 (83.3%)
Other legislation	752 (1.9%)	38,252 (96.9%)	461 (1.2%)
Total	**712,405 (54.1%)**	**550,313 (41.8%)**	**54,534 (4.1%)**

Table 23: Regulatory use by type of legislation in 2021

Legislation on medicinal products for human or veterinary uses is mainly related to quality controls. Industrial chemical legislation, medical devices legislation, food legislation including food contact

17 OJ L 285, 1.11.2017, p. 8–9

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

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

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 2021, for regulatory uses, 15.2% were reported as severe, 22.9% as moderate, 61.2% mild (and up to mild) and 0.7% as non-recovery. Even if the total numbers of uses are not the most significant in the area of food legislation including legislation for the safety of target animals, workers and environment (32%) and biocides (40.5%), the proportion of severe uses is relatively high and in the area of "Other' legislation", about 37.2% of procedures were reported as severe (Table 24).

	Non-recovery	Mild [up to and including]	Moderate	Severe
Legislation on medicinal products for human use	8,247 (1.2%)	391,316 (58.1%)	167,978 (24.9%)	105,868 (15.7%)
Legislation on medicinal products for veterinary use and their residues	60 (0%)	183,421 (60.7%)	66,572 (22%)	52,072 (17.2%)
Medical devices legislation	27 (0.1%)	32,268 (65.9%)	16,044 (32.8%)	593 (1.2%)
Industrial chemicals legislation	9 (0%)	124,328 (75.3%)	34,009 (20.6%)	6,740 (4.1%)
Plant protection product legislation	0 (0%)	35,159 (63%)	7,567 (13.6%)	13,072 (23.4%)
Biocides legislation	0 (0%)	1,190 (46.2%)	345 (13.4%)	1,043 (40.5%)
Food legislation including food contact material	0 (0%)	13,554 (84.3%)	1,143 (7.1%)	1,382 (8.6%)
Feed legislation including legislation for the safety of target animals, workers and environment	0 (0%)	3,906 (28.3%)	5,466 (39.7%)	4,408 (32%)
Other legislation	668 (1.7%)	21,487 (54.4%)	2,635 (6.7%)	14,675 (37.2%)
Total	**9,011 (0.7%)**	**806,629 (61.2%)**	**301,759 (22.9%)**	**199,853 (15.2%)**

Table 24: Severity of regulatory uses by type of legislation in 2021

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 (61.4%), rodents (29.8%) and birds (7.2%).

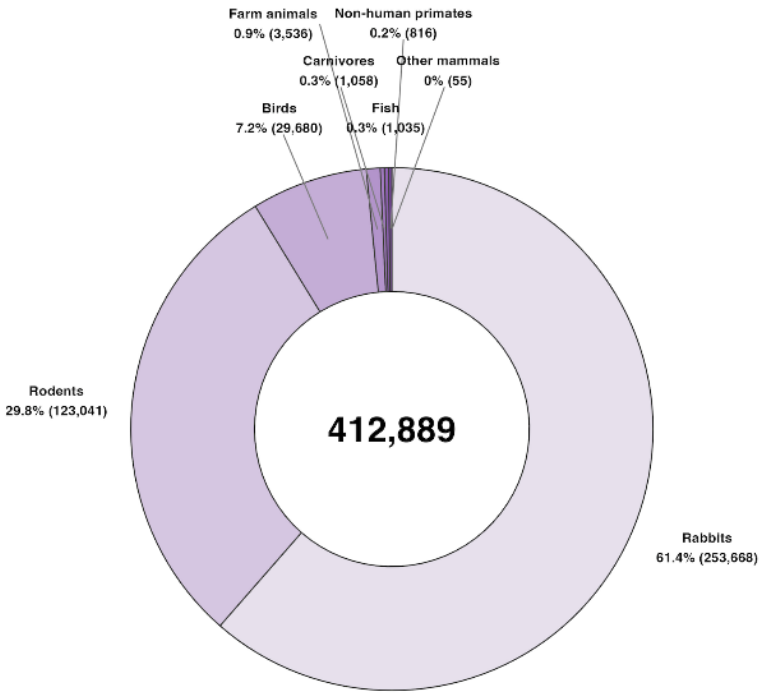


Figure 17: Main species used for routine production in 2021

In 2021, 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%).

To allow for a more detailed analysis on how the production of animal-derived antibodies is being replaced by non-animal derived antibodies, a further category covering mono- and polyclonal antibodies (excluding the use of mouse ascites method) was created, in addition to the already existing category for the production of monoclonal antibodies using mouse ascites method.

37.8% of routine uses were related to the production of blood-based products, 9% for monoclonal antibodies production by mouse ascites method, and 25.7% for the production of mono- and polyclonal antibodies (excluding mouse ascites method) (Figure 18).

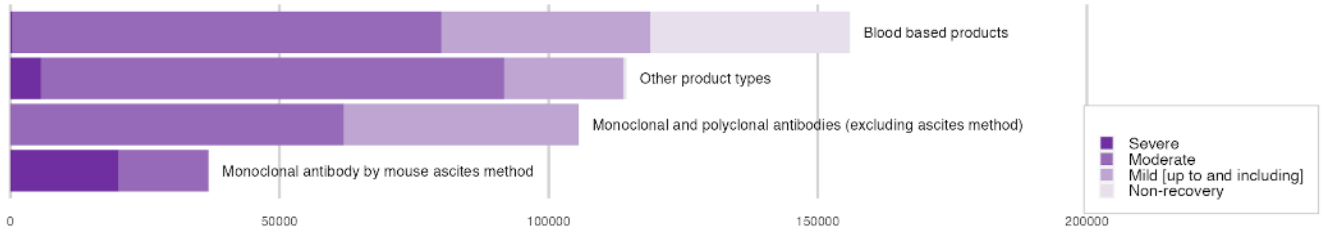


Figure 18: Routine production uses by product type and severity in 2021

While blood-based products involved only mild and moderate levels of severity, monoclonal antibody production by the mouse ascites method involved mostly severe uses (56%).

In 2021, monoclonal antibody production by the mouse ascites method, the most severe procedure (56% of severe uses), showed a decrease of -12% compared to 2020 still not showing a steady decrease compared since 2018 although it decrease by -32.9% compared to 2018. In total, only four Member States reported the use of the mouse ascites method for the production of monoclonal antibodies of which one Member State (France) represents 99.4%. In 2021 Germany decreased the number of uses by -92.8% (Table 25).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Belgium	11 (0%)	17 (0%)	0 (0%)	**0 (0%)**	NA	-100%
Czech Republic	93 (0.2%)	76 (0.2%)	125 (0.3%)	**75 (0.2%)**	-40%	-19.4%
France	53,073 (96.6%)	35,801 (95.5%)	40,649 (97%)	**36,664 (99.4%)**	-9.8%	-30.9%
Germany	1,565 (2.8%)	1,456 (3.9%)	1,056 (2.5%)	**113 (0.3%)**	-89.3%	-92.8%
Hungary	134 (0.2%)	0 (0%)	66[20] (0.2%)	**0 (0%)**	-100%	-100%
Poland	0 (0%)	4 (0%)	0 (0%)	**30 (0.1%)**	NA	NA
Spain	65 (0.1%)	119 (0.3%)	20 (0%)	**0 (0%)**	-100%	-100%
Total	**54,941 (100%)**	**37,473 (100%)**	**41,916 (100%)**	**36,882 (100%)**	**-12%**	**-32.9%**

Table 25: Number of uses for monoclonal antibody by mouse ascites method per country between 2018 and 2021

20 After the publication of 2020 data, Hungary reported that the 66 uses were misclassified, and no mouse ascites had been used in Hungary in 2020.

The production of mono- and polyclonal antibodies (excluding the mouse ascites method) was reported by 17 Member States 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 2021, the last five categories of uses reported as part of the Directive covered about 900,000 uses, a very important increase compared to the 2018-2020 average (+92.5%), mainly 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 (92.4% and 98%), while higher education and training for the acquisition, maintenance or improvement of vocational skills uses more mice (42.3% and 57.8%), rats (29.4% and 23.1%) and mammals (12.4% that 15.1%) (Table 26).

	Protection of the natural environment	Preservation of species	Higher education	Training for vocational skills	Forensic enquiries
Mice	1,001 (1.2%)	6,483 (8%)	18,558 (22.9%)	54,263 (66.9%)	788 (1%)
Rats	1,528 (4.2%)	123 (0.3%)	12,917 (35.6%)	21,729 (59.9%)	0 (0%)
Other mammals	4,689 (18.3%)	1,251 (4.9%)	5,442 (21.2%)	14,153 (55.1%)	134 (0.5%)
Fish	160,220 (21.6%)	574,038 (77.5%)	4,220 (0.6%)	2,016 (0.3%)	0 (0%)
Birds	4,343 (48.5%)	1,660 (18.6%)	1,908 (21.3%)	976 (10.9%)	60 (0.7%)
Amphibians, Cephalopods, Reptiles	1,675 (36.8%)	1,976 (43.4%)	535 (11.7%)	371 (8.1%)	0 (0%)
Dogs, Cats, NHPs	8 (1.1%)	0 (0%)	299 (40.2%)	437 (58.7%)	0 (0%)
Total	**173,464 (19.3%)**	**585,531 (65.2%)**	**43,879 (4.9%)**	**93,945 (10.5%)**	**982 (0.1%)**

Table 26: Main species used for “Other types of uses” in 2021

In 2021, with 585,531 uses in 2021, preservation of species is the biggest category of the remaining purposes, representing more than half of the other uses, a +452.1% increase compared to 2020. On the contrary, forensic inquiry uses are limited to about a thousand, but saw an important increase as well (+835.2% compared to 2020). (Figure 19).

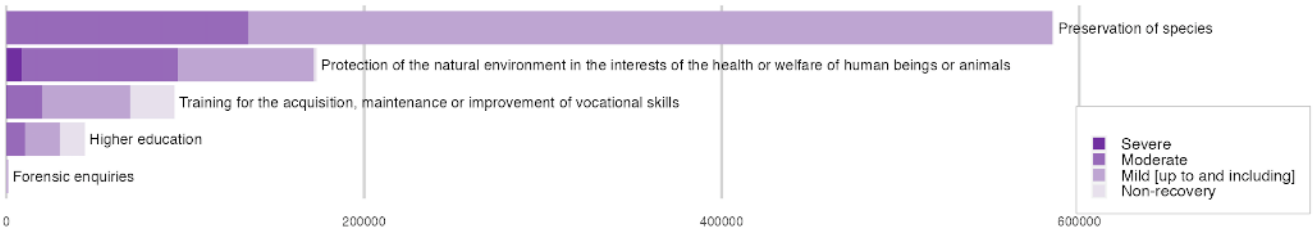


Figure 19: Other types of uses in 2021 including their severity

Lastly, the Directive update makes now a distinction between uses reported for higher education and for training for the acquisition, maintenance or improvement of vocational skills allowing more details.

Looking at the variation of these two categories in 2021 compared to 2018-2020, the number remained stable, despite a lower number in 2020, probably due to lockdowns preventing practical training taking place during the COVID-19 pandemic.

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 2021, the proportion of reuses was at 1.1% which represents a continuous decrease compared to 2018 both in absolute value (-31.7%) and proportion (-0.6%) (table 27).

	2018	2019	2020	2021	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%)**	+18.5%	+6.6%
Yes	156,677 (1.7%)	135,785 (1.6%)	116,866 (1.5%)	**106,981 (1.1%)**	-8.5%	-31.7%
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**	**+18.1%**	**+5.9%**

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

In proportion, procedures requiring the re-use of animals are related to regulatory use: skin irritation/corrosion (52.7%), eye irritation/corrosion (31.3%), pyrogenicity testing (13.4%); higher education (10.7%); and training for the acquisition, maintenance or improvement of vocational skills (8.5%).

In absolute numbers, the main procedures requiring re-use of animals were batch potency testing (12,365), animal nutrition (10,922), training for the acquisition, maintenance or improvement of vocational skills (8,028) and batch safety testing (6,596) (Figure 20).

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

	Percent	Number
Other species of Old World Monkeys (Cercopithecoidea)	100%	13
Squirrel monkey	60.6%	20
Vervets (Chlorocebus spp.)	57.1%	4
Cats	48.8%	1,925
Goats	44.7%	877
Dogs	35.8%	5,648
Rhesus monkey	33.5%	140
Horses, donkeys and cross-breeds	33.2%	1,545
Cynomolgus monkey	24.2%	1,513
Cattle	19.6%	5,384
Xenopus	15.6%	3,779
Sheep	13.4%	2,418
Reptiles	13.1%	367
Prosimians	7.8%	5
Marmoset and tamarins	7.1%	13
Other mammals	5.4%	278
Other carnivores	4.7%	107
Baboons	4.3%	2
Ferrets	3%	47
Pigs	2.8%	2,246
Other birds	1.8%	1,257
Rabbits	1.8%	6,708
Hamsters (Syrian)	1.7%	476
Other fish	1.3%	5,690
Rats	1.2%	8,278
Cephalopods	1.1%	33
Guinea-Pigs	1.1%	1,228
Mice	1.1%	47,113
Other rodents	1%	175
Guppy, swordtail, molly, platy	0.9%	789
Sea bass	0.8%	5,101
Mongolian gerbil	0.6%	21
Zebra fish	0.5%	1,633
Domestic fowl	0.3%	1,134
Other amphibians	0.2%	35
Salmon, trout, chars and graylings	0.1%	961
Turkey	0.1%	18
Hamsters (Chinese)	0%	0
Rana	0%	0

Table 28: Reuses by type of species in 2021

In proportion, non-human primates are reused more often than other species: cercopithecoidea (100%), squirrel monkey (60.6%), vervets (57.1%), rhesus monkeys (33.5%), cynomolgus monkeys (24.2%); however, in limited numbers. Other animals frequently reused in proportion are large mammal species such as cats (48.8%), goats (44.7%), dogs (35.8%), horses, donkeys and cross-breeds (33.2%), cattle (19.6%) or sheep (13.4%); in larger numbers. Xenopus (15.6%) and reptiles (13.1%) are also re-used, to a lower extent.

In absolute numbers, the main species reused for scientific purposed in 2021 were mice (47,113), rats (8,278), rabbits (6,708), dogs (5,648) and cattle (5,384) (Table 28).

According to the Directive, reuse of an animal is not allowed in a procedure classified prospectively as severe. In 2021, most of the reuses, the actual reported severities were mild (68.6%) or moderate (28.3%) (Figure 21).

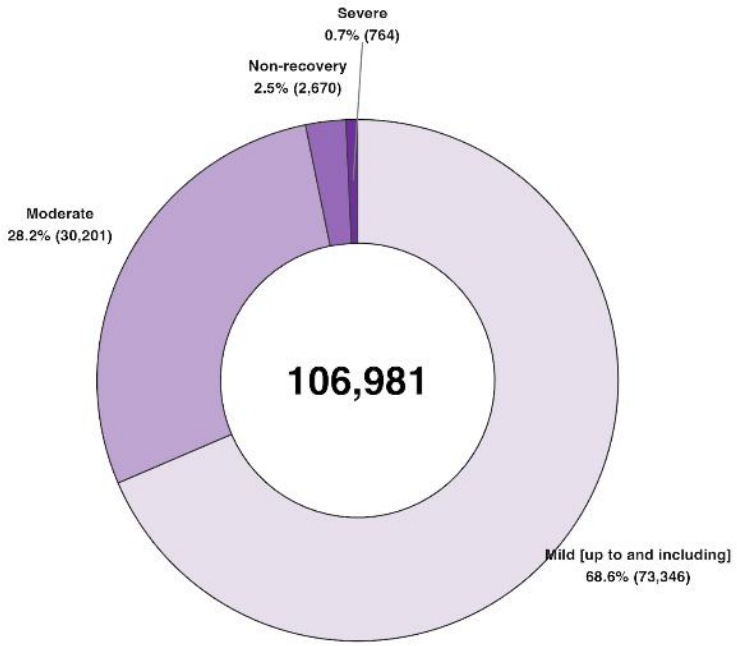


Figure 21: Reuses by severity in 2021

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 764 such cases compared to 2020.

These 764 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
Mild [up to and including]	107,781 (68.8%)	99,518 (73.3%)	83,574 (71.5%)	**73,346 (68.6%)**
Moderate	36,053 (23%)	32,921 (24.2%)	30,946 (26.5%)	**30,201 (28.2%)**
Non-recovery	12,762 (8.1%)	3,022 (2.2%)	1,733 (1.5%)	**2,670 (2.5%)**
Severe	81 (0.1%)	324 (0.2%)	613 (0.5%)	**764 (0.7%)**
Total	**156,677 (100%)**	**135,785 (100%)**	**116,866 (100%)**	**106,981 (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 2021, 1.95 million uses for the purposes of research were carried out on animals that were genetically altered. Of these, 16.9% were carrying a harmful phenotype (Figure 22).

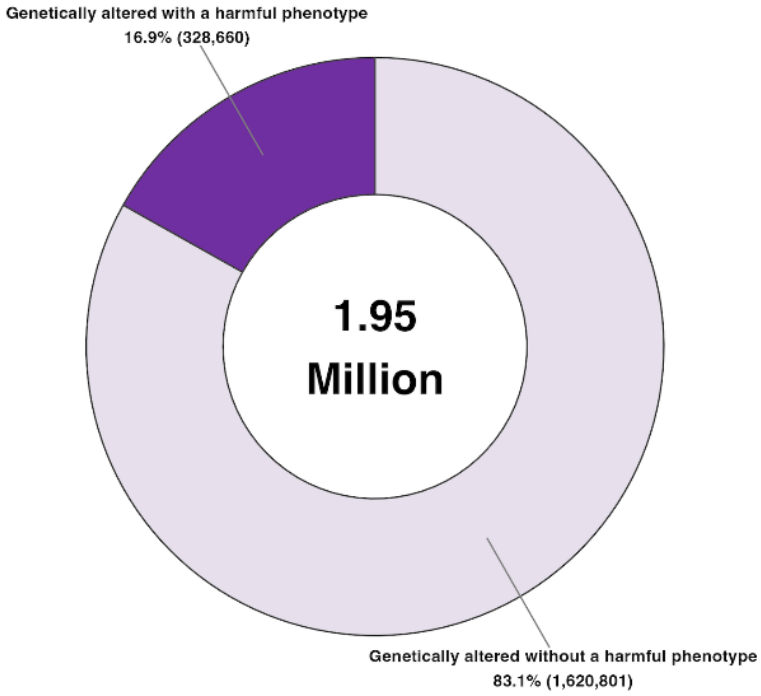


Figure 22: Uses of animals by type of genetic alteration in 2021

In 2021, the proportion of the uses of genetically altered animals for scientific purposes remained stable around 20%. The percentage of the uses of such animals without a harmful phenotype remained stable at 17%, as well as the uses of such animals with a harmful phenotype at 3.5% (Table 30).

	2018	2019	2020	2021
Genetically altered with a harmful phenotype	275,660 (3.1%)	326,789 (3.7%)	313,937 (3.9%)	328,660 (3.5%)
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%)
Not genetically altered	7,184,621 (80%)	6,797,565 (78%)	6,255,328 (77.7%)	7,563,753 (79.5%)
Total	**8,979,081 (100%)**	**8,715,224 (100%)**	**8,054,930 (100%)**	**9,513,214 (100%)**

Table 30: Genetic status of animals used in 2021

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, 49.2% of zebra fish was genetically altered, followed by mice (41.7%), while only 3.7% of rats were genetically altered used in procedures for purposes of research, testing, routine production in 2020 (Table 31).

There is no evident trend in the evolution since 2018 but it seems that the proportion of genetically altered mice (from 39.1% to 41.7%) and other amphibians (from 10.1% to 32%) is increasing while the proportion genetically altered rabbits (from 7.3% to 5.4%) and xenopus (from 20.1% to 2.6%) is decreasing.

	2018	2019	2020	2021
Mice	39.1%	41.5%	40.6%	**41.7% (1,724,503)**
Rats	3.4%	3.1%	3.4%	**3.7% (25,494)**
Hamsters (Syrian)	2.5%	4.6%	0%	**0.8% (226)**
Other rodents	0%	0.1%	0%	**0% (0)**
Rabbits	7.3%	6%	5.1%	**5.4% (19,504)**
Dogs	0.4%	0.3%	0.1%	**0.1% (9)**
Ferrets	0%	0.5%	3%	**0% (0)**
Pigs	0.5%	0.8%	0.8%	**1% (841)**
Sheep	0%	0%	0%	**0.3% (60)**
Prosimians	0%	0%	0%	**23.4% (15)**
Domestic fowl	0.1%	0.1%	0%	**0.1% (317)**
Xenopus	20.1%	13.3%	7.4%	**2.6% (644)**
Other amphibians	10.1%	17.9%	27.4%	**32% (5,311)**
Zebra fish	59.9%	62.7%	52.4%	**49.2% (168,328)**
Other fish	0.2%	0.2%	0.3%	**0.7% (3,001)**

Table 31: Genetically altered species used between 2018 and 2021

Genetically altered animals are used almost exclusively for research purposes. In 2021, the proportion of genetically altered animals was the highest in basic research for musculoskeletal system (67.3%), cardiovascular, blood and lymphatic system (65.8%), developmental biology (64%), sensory organs (57.2%), nervous system (53.2%), endocrine system/metabolism (52.8%), oncology (51.9%); in translational and applied research to a lower extent for human musculoskeletal disorders (65.6%), human cancer (39.2%), other human disorders (34.9%), human endocrine/metabolism disorders (38.3%); and in training for the acquisition, maintenance or improvement of vocational skills (20%) (Figure 23).

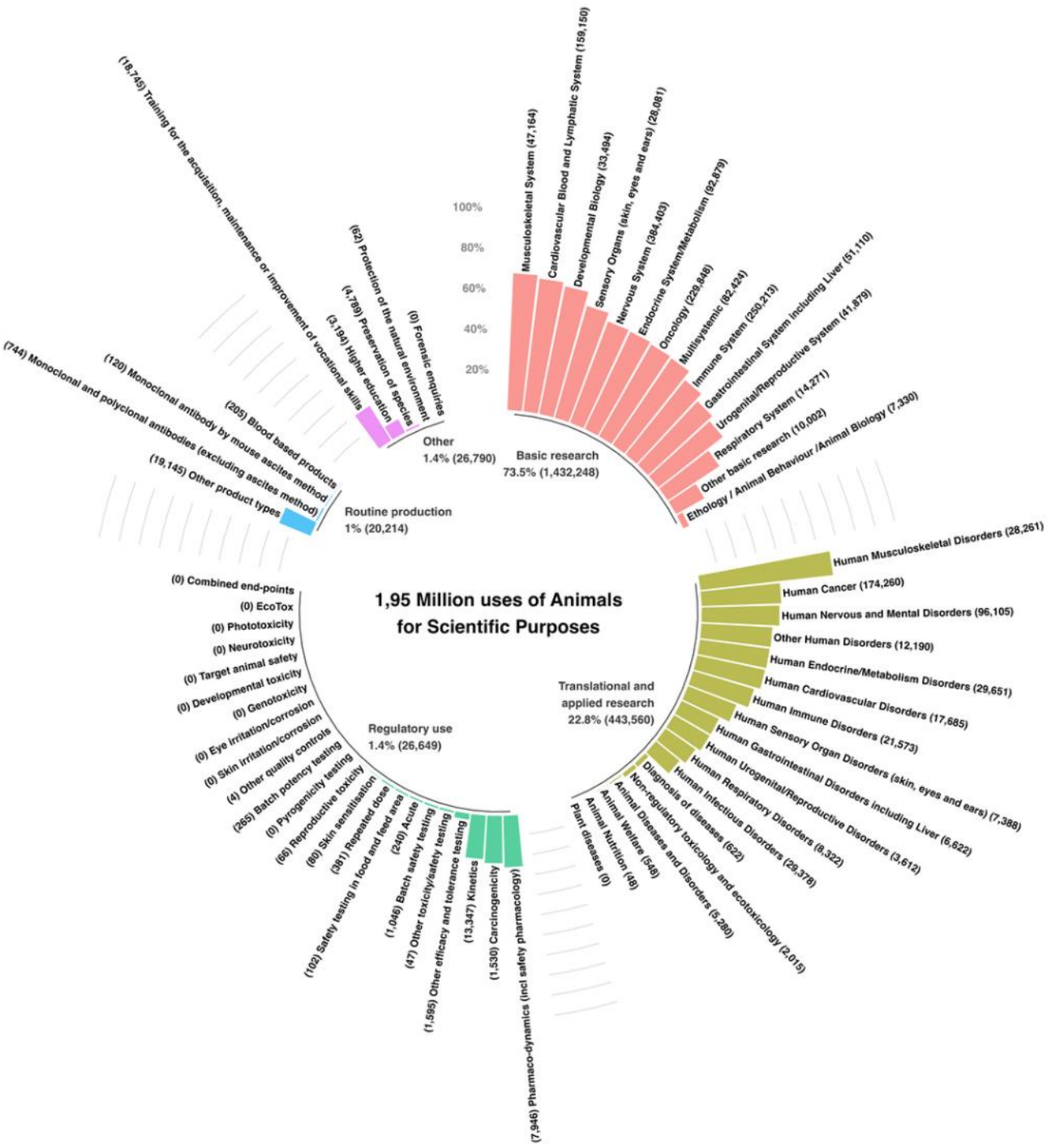


Figure 23: Proportion of genetically altered animals uses by purpose in 2021

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.

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 2021, 664,965 animals were used for the provision of genetically altered animals for the purposes of scientific research.

This included 356,706 animals used for the first time for the creation of new genetically altered animal lines (Table 32), which remains stable compared to the 2018-2020 reporting years.

360,478 animals were used for the first time for the maintenance of colonies of established genetically altered animal lines (Table 32). In comparison to 2020, this represents a decrease of -8.4%. It is important to note in this context that the reporting requirements for the maintenance of colonies of established genetically altered animal lines are particularly complex. The Commission, Member States and key stakeholder organisations have been working to provide more guidance to the users. A guidance document on genetically altered animals was endorsed by Member State National Contact Points responsible for the implementation of the Directive in their meeting in November 2021[4]. More information is provided under section IV.3.3 below.

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. (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% and 26.1% respectively. Other species, although in small numbers, include other species of fish, rats, Salmon, trout, chars and graylings (Table 32).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Mammals						
Rodents						
Mice	274,354	214,078	293,821	**255,978**	-12.9%	-6.7%
Rats	6,168	3,635	5,138	**1,938**	-62.3%	-68.6%
Guinea-Pigs	0	0	0	**0**	NA	NA
Hamsters (Syrian)	89	116	449	**270**	-39.9%	+203.4%
Other rodents	0	0	0	**0**	NA	NA
Rabbits						
Rabbits	324	305	277	**392**	+41.5%	+21%
Carnivores						
Dogs	0	0	18	**0**	-100%	NA
Ferrets	4	0	0	**0**	NA	-100%
Other carnivores	0	0	6	**0**	-100%	NA
Farm animals						
Horses, donkeys and cross-breeds	0	0	10	**0**	-100%	NA
Pigs	62	124	131	**385**	+193.9%	+521%
Goats	0	0	0	**0**	NA	NA
Sheep	0	0	263	**0**	-100%	NA
Cattle	0	1	28	**0**	-100%	NA
Non-human primates						
Marmoset and tamarins	10	47	0	**0**	NA	-100%
Other mammals						
Other mammals	70	6	10	**0**	-100%	-100%
Birds						
Domestic fowl	100	286	293	**525**	+79.2%	+425%
Other birds	0	10	183	**36**	-80.3%	NA
Reptiles						
Reptiles	0	0	42	**0**	-100%	NA
Amphibians						
Xenopus	0	1,748	819	**961**	+17.3%	NA
Other amphibians	100	271	368	**805**	+118.8%	+705%
Fish						
Zebra fish	84,996	89,392	89,787	**94,186**	+4.9%	+10.8%
Other fish	16,087	3,122	2,103	**3,248**	+54.4%	-79.8%
Salmon, trout, chars and graylings	0	0	0	**1,754**	NA	NA
Totals						
Total	**382,364**	**313,141**	**393,746**	**360,478**	**-8.4%**	**-5.7%**

Table 32: Uses of animals for the creation of new genetically altered animal lines by species between 2018 and 2021

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 lines 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, 64.8% were of a non-harmful phenotype.

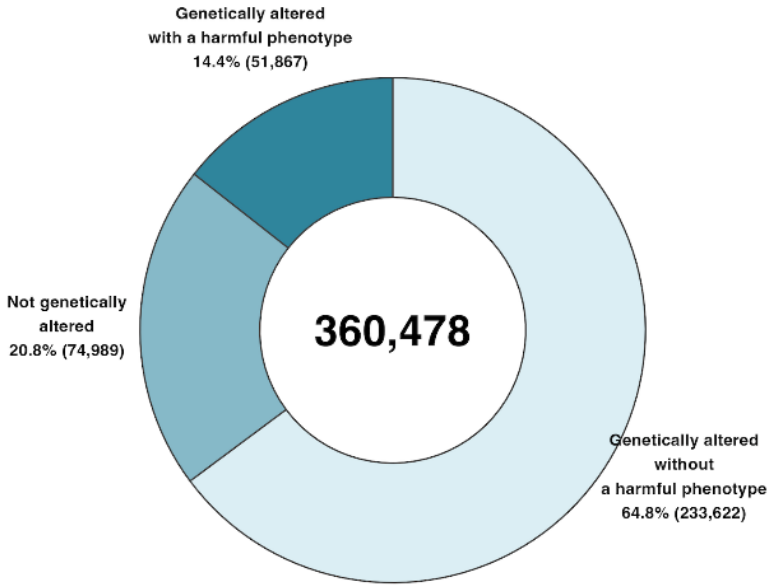


Figure 24: Creation of new genetically altered animal lines: genetic types of animal used in 2021

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 2021, 360,478 uses (first and any subsequent reuses) were reported for the purposes of creating new genetically altered animal lines (Figure 25).

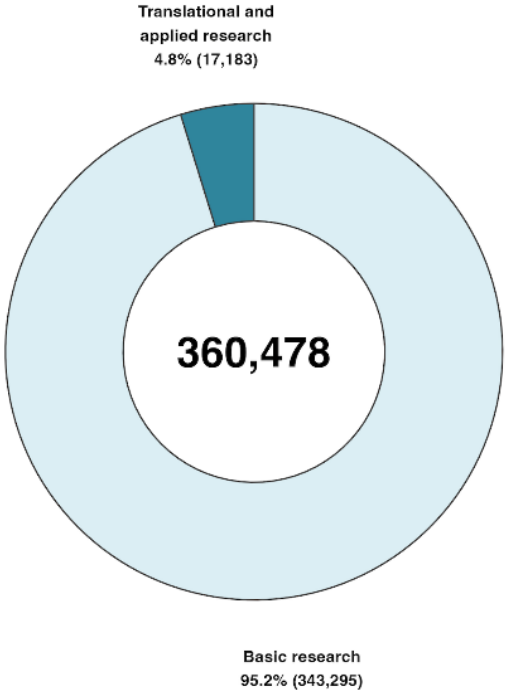


Figure 25: Creation of new genetically altered animal lines: uses for research purposes in 2021

95.2% 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 2021, basic research purposes concerned of uses for multisystemic (29.7%), cardiovascular, blood and lymphatic system (13.5%), nervous system (13.4%) and oncology (10.5%). The most important sub-category under translational and applied research for which new genetically altered animal lines were created was human endocrine/metabolism disorders (0.8%).

	2018	2019	2020	2021	Change 2021 vs 2020	Change 2021 vs 2018
Basic research						
Oncology	50,180 (13.1%)	38,803 (12.4%)	43,817 (11.1%)	**37,678 (10.5%)**	-14%	-24.9%
Cardiovascular Blood and Lymphatic System	42,560 (11.1%)	41,118 (13.1%)	43,422 (11%)	**48,546 (13.5%)**	+11.8%	+14.1%
Nervous System	63,676 (16.7%)	53,772 (17.2%)	82,050 (20.8%)	**48,432 (13.4%)**	-41%	-23.9%
Respiratory System	510 (0.1%)	200 (0.1%)	521 (0.1%)	**489 (0.1%)**	-6.1%	-4.1%
Gastrointestinal System including Liver	9,283 (2.4%)	12,091 (3.9%)	13,739 (3.5%)	**12,066 (3.3%)**	-12.2%	+30%
Musculoskeletal System	11,068 (2.9%)	6,259 (2%)	7,936 (2%)	**5,784 (1.6%)**	-27.1%	-47.7%
Immune System	33,529 (8.8%)	26,794 (8.6%)	25,299 (6.4%)	**34,146 (9.5%)**	+35%	+1.8%
Urogenital/Reproductive System	22,100 (5.8%)	20,606 (6.6%)	15,246 (3.9%)	**10,040 (2.8%)**	-34.1%	-54.6%
Sensory Organs (skin, eyes and ears)	10,957 (2.9%)	8,172 (2.6%)	3,637 (0.9%)	**4,815 (1.3%)**	+32.4%	-56.1%
Endocrine System/Metabolism	17,086 (4.5%)	11,787 (3.8%)	16,411 (4.2%)	**18,219 (5.1%)**	+11%	+6.6%
Developmental Biology	0 (0%)	0 (0%)	0 (0%)	**9,776 (2.7%)**	NA	NA
Multisystemic	59,131 (15.5%)	55,062 (17.6%)	71,956 (18.3%)	**106,912 (29.7%)**	+48.6%	+80.8%
Ethology / Animal Behaviour /Animal Biology	2,388 (0.6%)	1,410 (0.5%)	1,363 (0.3%)	**1,558 (0.4%)**	+14.3%	-34.8%
Other basic research	23,830 (6.2%)	22,099 (7.1%)	13,257 (3.4%)	**4,834 (1.3%)**	-63.5%	-79.7%
Translational and applied research						
Human Cancer	3,221 (0.8%)	2,562 (0.8%)	3,048 (0.8%)	**2,189 (0.6%)**	-28.2%	-32%
Human Infectious Disorders	1,062 (0.3%)	84 (0%)	2,456 (0.6%)	**1,883 (0.5%)**	-23.3%	+77.3%
Human Cardiovascular Disorders	2,298 (0.6%)	1,844 (0.6%)	1,619 (0.4%)	**1,489 (0.4%)**	-8%	-35.2%
Human Nervous and Mental Disorders	3,156 (0.8%)	2,467 (0.8%)	4,036 (1%)	**1,661 (0.5%)**	-58.8%	-47.4%
Human Respiratory Disorders	608 (0.2%)	35 (0%)	377 (0.1%)	**178 (0%)**	-52.8%	-70.7%
Human Gastrointestinal Disorders including Liver	3,224 (0.8%)	344 (0.1%)	443 (0.1%)	**664 (0.2%)**	+49.9%	-79.4%
Human Musculoskeletal Disorders	317 (0.1%)	217 (0.1%)	430 (0.1%)	**431 (0.1%)**	+0.2%	+36%
Human Immune Disorders	429 (0.1%)	769 (0.2%)	491 (0.1%)	**1,817 (0.5%)**	+270.1%	+323.5%
Human Urogenital/Reproductive Disorders	171 (0%)	122 (0%)	207 (0.1%)	**954 (0.3%)**	+360.9%	+457.9%
Human Sensory Organ Disorders (skin, eyes and ears)	783 (0.2%)	729 (0.2%)	894 (0.2%)	**862 (0.2%)**	-3.6%	+10.1%
Human Endocrine/Metabolism Disorders	10,551 (2.8%)	2,434 (0.8%)	1,726 (0.4%)	**2,706 (0.8%)**	+56.8%	-74.4%
Other Human Disorders	820 (0.2%)	3,018 (1%)	38,953 (9.9%)	**1,916 (0.5%)**	-95.1%	+133.7%
Animal Diseases and Disorders	9,169 (2.4%)	295 (0.1%)	157 (0%)	**357 (0.1%)**	+127.4%	-96.1%
Animal Welfare	223 (0.1%)	0 (0%)	127 (0%)	**0 (0%)**	-100%	-100%
Diagnosis of diseases	0 (0%)	20 (0%)	87 (0%)	**9 (0%)**	-89.7%	NA
Non-regulatory toxicology and ecotoxicology	34 (0%)	28 (0%)	41 (0%)	**67 (0%)**	+63.4%	+97.1%
Total	**382,364 (100%)**	**313,141 (100%)**	**393,746 (100%)**	**360,478 (100%)**	**-8.4%**	**-5.7%**

Table 33: Uses of animals for the creation of new genetically altered animal lines by type of research between 2018 and 2021

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.

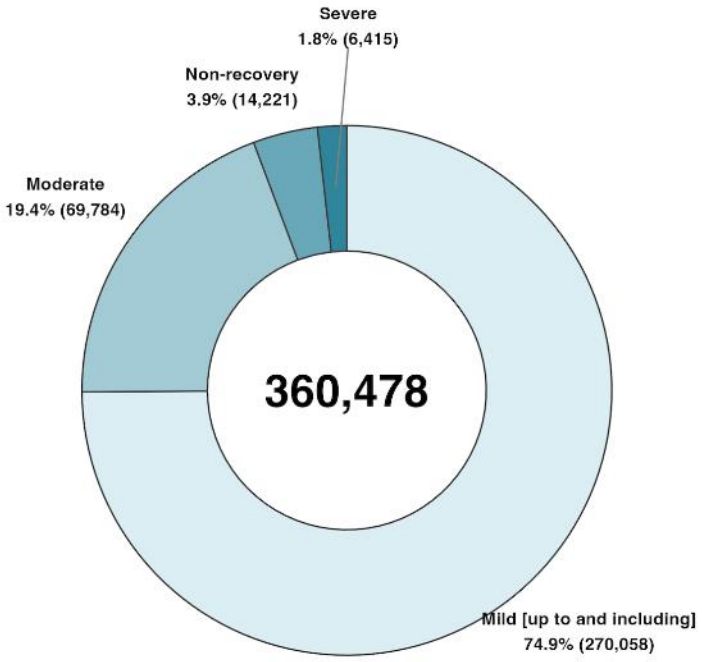


Figure 26: Uses of animals for the creation of new genetically altered animal lines by severities in 2021

IV.3.2.4. Reuses

In 2021, the number of reuses for the creation of new genetic lines was 1% (Table 34).

	Yes	No
Mice	0.6% (1,566)	99.4% (254,412)
Rats	0% (0)	100% (1,938)
Hamsters (Syrian)	0% (0)	100% (270)
Rabbits	0.3% (1)	99.7% (391)
Pigs	2.3% (9)	97.7% (376)
Domestic fowl	0% (0)	100% (525)
Other birds	0% (0)	100% (36)
Xenopus	0% (0)	100% (961)
Other amphibians	0% (0)	100% (805)
Zebra fish	2.3% (2,196)	97.7% (91,990)
Other fish	0% (0)	100% (3,248)
Salmon, trout, chars and graylings	0% (0)	100% (1,754)
Total	**1% (3,772)**	**99% (356,706)**

Table 34: Reuse of animals used for the creation of new genetically altered animal lines in 2021

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 2021, 315,828 uses were reported under the maintenance of colonies of established genetically altered animal lines. Amongst these uses, 65.4% were genetically altered without a harmful phenotype, 27.8% with a harmful phenotype and 6.7% without genetic alteration (Figure 27).

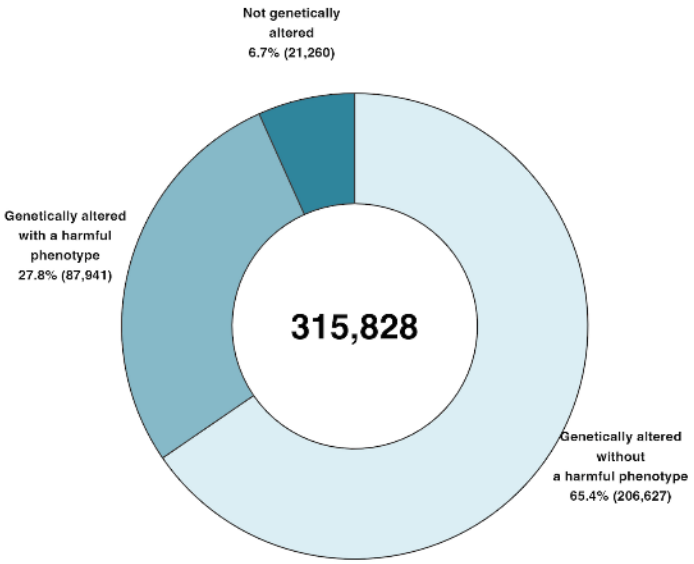


Figure 27: Genetic status of animals used for the maintenance of colonies of established genetically altered animal lines in 2021

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

In 2021, 77.6% 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 65.4% 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'.

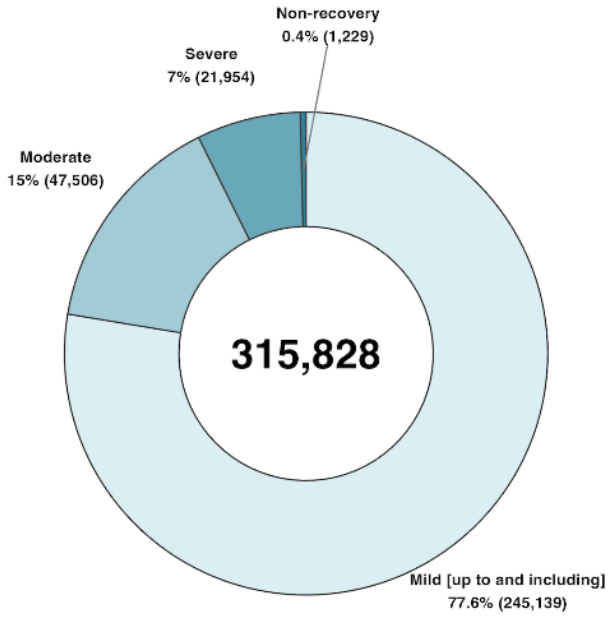


Figure 28: Uses of animals for the maintenance of colonies of genetically altered animal lines by severity in 2021

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	Change 2021 vs 2020	Change 2021 vs 2018
Mammals						
Rodents						
Mice	518,180	336,427	292,513	**279,911**	-4.3%	-46%
Rats	3,827	3,935	8,214	**10,193**	+24.1%	+166.3%
Carnivores						
Dogs	5	10	14	**7**	-50%	+40%
Farm animals						
Pigs	0	0	92	**0**	-100%	NA
Birds						
Domestic fowl	219	0	0	**0**	NA	-100%
Fish						
Zebra fish	7,840	11,520	2,001	**25,144**	+1156.6%	+220.7%
Other fish	1,262	911	1,794	**573**	-68.1%	-54.6%
Totals						
Total	**531,333**	**352,803**	**304,628**	**315,828**	**+3.7%**	**-40.6%**

Table 35: Uses of animals for the maintenance of colonies of established genetically altered animal lines by species between 2018 and 2021

IV.3.3.3. Reuses

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

	Yes	No
Mice	2.3% (6,473)	97.7% (273,438)
Rats	0% (0)	100% (10,193)
Dogs	0% (0)	100% (7)
Zebra fish	4.4% (1,096)	95.6% (24,048)
Other fish	0% (0)	100% (573)
Total	**2.4% (7,569)**	**97.6% (308,259)**

Table 36: Reuses by species for the maintenance of colonies of established genetically altered animal lines in 2021

V. MEMBER STATE NARRATIVES 2021

Introduction

Member States submitted 2021 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 2021, and Norway.

The Member State data is available through public ALURES Statistical EU Database[21]

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 2021 which can be found in this section of the EU summary report.

21 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 2021 is 218.244 (2020: 206.469) which is an increase of approximately 5,7% or in absolute numbers 11.775 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.

An increase is especially observed in the total number of zebrafish used for scientific purposes from 11.710 animals in 2020 to 24.463 animals in 2021 whereas the total number of mice used for scientific purposes decreased from 168.995 in 2020 to 165.884 animals in 2021. Zebrafish were mainly used in “Basic research, Multisystemic” and “Basic research, developmental biology”.

(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:

An increase is observed in “Basic Research, Multisystemic” (from 15.441 in 2020 to 25.104 animals in 2021) as well as “Translational and Applied Research, Human Endocrine/Metabolism Disorders” (from 809 in 2020 to 1.812 animals in 2021).

Whereas a decrease is registered in “Basic Research, Oncology” (from 28.161 to 22.856 animals) and “Translational and Applied Research, Human Infectious Disorders (from 34.462 to 28.687 animals).

There is an increase in “Maintenance of colonies of established genetically altered animals, not used in procedures” from 2.284 animals in 2020 to 5.566 animals in 2021.

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

The proportion of severity "severe" decreased slightly from 11% to 10% compared to the previous year (from 22.137 animals to 21.968 animals) whereas an increase is observed in “non-recovery” (from 4.825 animals to 5.408 animals).

(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.

The Austrian Government promotes projects to further advance the development of alternative approaches which could use fewer animals or which entail less painful procedures or which do not involve the use of animals at all. The establishment of the national 3Rs center is supported to promote alternative approaches and to disseminate information and implement 3R best practice. In addition, the Austrian Government reintroduced an award that acknowledges and honours outstanding scientific projects 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" (92 animals) include i.a. Barbastella barbastellus (38%), Lepus europaeus (26%), Cervus Elephalus (14%),

"Other birds" (949 animals) include i.a. Parus major (22%) and Cyanistes caeruleus (15%), Columba livia f. domestica (14%), Acrocephalus scirpaceus (12%),

"Other fish" (6448 animals) include i.a. Alburnus alburnus (21%), Rutilus rutilus (16%), Squalius cephalus (8%), Chondrostoma nasus (8%) and

"Other amphibian" (5316 animals) include mainly Ambystoma mexicanum (98%).

(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 neither authorized nor reported.

Belgium

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

In 2021 there is an increase of 9,24% compared to 2020. If we compare the number with 2019, we see a decrease of 3,30%. And compared to 2018 there is a decrease of 14,13%. We can assume that the observed decrease in 2020 was, at least partially, caused by the measures taken because of the Covid19 pandemic. In 2021, the increase is at least partially due to the revival of activities that were previously shut down or limited.

Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
556271	493982	437275	477675

Since 2015, the number of re-use continues to decline. Compared to 2020 there is a decrease of 5,05% and even a decrease of 27,33% compared to 2019.

Re-Use	Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
No	551601	489814	434085	474646
Yes	4670	4168	3190	3029
Total uses	**556271**	**493982**	**437275**	**477675**

Over the years, we see a gradual decline in the number of mammals that are used in procedures.
The use of birds fluctuates and is higher in 2021 than in the previous three years.
The use of fish also fluctuates and had a sharp decline in 2020. In 2021, this figure is similar to 2018.
The use of amphibians and reptiles is decreasing over the years.

Species	Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
Mammals	454576	401065	362087	364534
Birds	45412	41703	45946	59749
Fish	54843	49807	28126	53035
Amphibians	1116	1106	1011	321
Reptiles	324	301	105	36

Total uses	**556271**	**493982**	**437275**	**477675**

Within the mammal category we noticed that the upward trend in the use of mice came to an end in 2019. In 2020 the use of mice dropped even further, but in 2021 there was a slight increase again. Considered over several years, the downward trend still persists. We see a similar trend in the use of rats.

There is a noteworthy increase in procedures on hamsters in recent years. The number of tests on hamsters has increased from 886 in 2019 to 2985 in 2020 and to 4744 in 2021. This is largely due to research conducted to develop a vaccine and drugs against the SARS-CoV-2 virus. Hamsters were used in this type of research because the species is susceptible to corona infections.

The decrease in rabbit use is partly due to the end of a specific project investigating group housing in rabbit farming in 2020 and by the growth in the use of non-animal methods in antibody production.

The decrease in cattle use is also largely explained by the end of a specific project that was reported in 2020 in which the genetic diversity of old cattle breeds was mapped by taking a small ear biopsy of a large number of farm animals.

There was an increase in the use of horses in 2021. These are mainly horses from private owners. The procedures involve minimally invasive examinations such as ultrasound, ECG and other heart rate monitoring and blood sampling.

Animal Species	Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
Mice	348937	299038	251913	267161
Rats	20003	17471	13760	14542
Hamsters	772	886	2985	4744
Rabbits	61575	63094	70761	61104
Cattle	850	1420	2329	1044
Horses, donkeys and cross-breeds	302	110	199	347

In 2021, the new reporting category for turkeys was used for the first time. This explains the decrease in the 'other birds' category in 2021.

We see increased use in the domestic fowl category. The increase is due to a greatly increased use of chickens in studies related to diseases and disorders in animals. Specifically, these are studies in which the safety and efficacy of drugs, anti-parasitic products and vaccines are investigated in farm animals. This also applies to the use of turkeys.

Animal Species	Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
Domestic fowl	39203	35292	41115	54511
Turkey				2473
Other birds	6209	6411	4831	2765

In 2021, the new reporting categories for 'guppy, swordtail, molly, platy' and 'salmon, trout, chars and graylings' were used for the first time. Nevertheless, there is no decrease but an increase in the 'other fish' category. This increase is mainly due to increased use of *Cyprinus carpio* (+1165), *Anguilla anguilla* (+1033) and *Rutillus rutillus* (+1020). Animals in the 'other fish' category are used for various purposes. These include species conservation studies and studies related to animal or human diseases or studies on animal biology or behaviour.

In 2020, there was a sharp decrease in animal studies involving zebrafish compared to 2019. This decrease was due to measures taken due to the COVID-19 epidemic. In 2021, measures were less stringent and delayed trials were started or continued.

Animal Species	Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
Zebra fish	25904	39115	22804	43113
Guppy, swordtail, molly, platy				350
Salmon, trout, chars and graylings				861
Other Fish	28939	10692	5322	8711

(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 has been declining in recent years. In 2021 it shows a slight increase again, probably due to the restart of activities that were restricted in 2020.
For basic research, the increase is 13,78%. For translational and applied research, it is a 27,91% increase.

The 'preservation of species' category drastically increased in 2021 in comparison with 2020. It mainly involves fish, and to a lesser extent birds and amphibians. For fish, it mainly concerns a trial where a large number of animals were used in a study on the harmfulness of pumps and turbine screws. Most of the animals were released back into the wild afterwards. In the case of birds, these were wild partridges used for genetic research. All these animals were freed immediately after blood sampling.

Purpose Category	Number of use in 2018	Number of use in 2019	Number of use in 2020	Number of use in 2021
Basic Research	251704	222946	168821	192085
Translational and applied research	121645	130724	120505	154134
Regulatory use and Routine production	140896	115267	127262	109548
Protection of the natural environment in the interests of the health or welfare of human beings or animals	359	798	743	58
Preservation of species	5598	243	371	3017
Higher education or training for the acquisition, maintenance or improvement of vocational skills	7442	6287	3827	4073
Forensic enquiries	0	0	0	0
Maintenance of colonies of established genetically altered animals, not used in other procedures	28627	17717	15746	14760
Non-EU Purpose	0	0	0	0
Total uses	**556271**	**493982**	**437275**	**477675**

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

Proportionally, the share of non-recovery procedures and the share of severe procedures are almost the same as in 2020.

In recent years, a trend was observed within the severity category of mild and moderate with a decrease of mild procedures and an increase in moderate procedures. This was confirmed in 2021. In 2021, this can partially be explained by an increasing number of procedures in the translational

and applied categories 'animal diseases and disorders' and 'human nervous and mental disorders'. These are two categories in which we see a large proportion of moderate procedures.

Severity	Number of use in 2018	%	Number of use in 2019	%	Number of use in 2020	%	Number of use in 2021	%
Non-recovery	20565	3,70%	14074	2,85%	9464	2,16%	11044	2,31%
Mild	311660	56,03%	284376	57,57%	241487	55,23%	241281	50,51%
Moderate	154633	27,80%	131963	26,71%	139284	31,85%	174492	36,53%
Severe	69413	12,48%	63569	12,87%	47040	10,76%	50858	10,65%
Total uses	556271	100,00%	493982	100,00%	437275	100,00%	477675	100,00%

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

Continuation of the RE-Place project (database that brings together expertise on alternative methods for animal testing) and funding of several specific 3R research projects.
In the Flemish region, round tables were initiated with a broad group of stakeholders. By 2023, these discussions should lead to an action plan with actions that can help reduce the number and share of animal experiments in the region and beyond.

A grant project has been set up for the years 2022, 2023 and 2024 with the aim to ensure, for the first time, the pooling of equipment and skills of the entire French-speaking scientific community of Belgium around the implementation of alternative methods to animal experimentation.

In the Brussels Capital Region grants were awarded for the creation of a Brussels platform for 3R alternatives. This platform will provide the Brussels Capital Region with a competent center to promote animal welfare and the application of the 3R Principle, 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. Since the attribution of the grant happened only recently, no impact on statistics is yet noticeable.

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

1. Other fish

   16.43% of the fishes are reported under the "other" category.
   They are mainly *Cyprinidae* (52%) and *Anguillidae* (24%), followed by *Nothobranchiidae* (9%), *Rivulidae* (8%) and *Cichlidae* (4%).

Other fish	Number of uses
Cyprinus carpio	2884
Anguilla anguilla	2062
Rutillus rutillus	1020

Kryptolebias marmoratus	715
Carassius	650
Nothobranchius furzeri	781
Cichlidae	191
Sander lucioperca	120
Oreochromis niloticus	81
Maylandia estherae	63
Alosa fallax	37
Haplochromis	16
Pseudotropheus saulosi	16
Dicentrarchus labrax	15
Parophidion vassali	10
Squalius cephalus	10
Pygocentrus nattereri	9
Diancistrus fuscus	6
Synodontis grandiops	6
Piaractus brachypomus	5
Esox lucius	4
Priacanthus hamrur	3
Serrasalmus maculatus	3
Colossoma macropomum	1
Gadus morhua	1
Lampetra fluviatilis	1
Silurus glanis	1

2. Other amphibians

33,96% of the amphibians are reported under the "other" category.
They are Alytidae (*Discoglossus pictus*, 69%) and Ranidae (*Lithobates catesbeianus*, 27%) and Salamandridae (*Ichtyosaura alpestris*, 4%).

Other amphibians	**Number of uses**
Discoglossus pictus	75
Lithobates catesbeianus	30
Ichtyosaura alpestris	4

3. Other birds

4,63% of the birds are reported under the "other" category.
They are mostly Numididae (41% of other birds) and Phasianidae (*Alectoris rufa*, *Coturnix coturnix*, *Coturnix japonica* and *Perdix perdix*) (40%). The other birds are members of Laridae (*Larus fuscus* and *Larus argentatus*) (7%), Fringillidae *(Serinus canaria)* (4%), Anatidae (3%),

Estrildidae (*Amandava amandava*, *Estrilda astrild*, *Estrilda troglodytes* and *Taeniopygia*) (2%) and Columbidae (2%).

Other birds	Number of uses
Numididae	1146
Alectoris rufa	535
Coturnix japonica	223
Coturnix coturnix	190
Perdix perdix	169
Serinus canaria	108
Larus argentatus	100
Larus fuscus	100
Anatidae	87
Columbidae	48
Amandava amandava	20
Estrilda astrild	20
Estrilda troglodytes	17
Taeniopygia	2

(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:

82 animals were used in the category 'Monoclonal and polyclonal antibodies (excluding ascites)', including 33 mice, 45 rats, 2 *Llama Glama* and 2 *Vicugna pacos*. In vitro methods for the production of monoclonal antibodies have been validated by the EURL ECVAM but animals are still used for their production in Belgium. 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 in which 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 2021, compared to 2020 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 covid pandemic and the online sessions in the universities. The used animals are only animals born in the EU at register breeder. The number of used rodents and dogs is increased, the number of used rabbits and artiodactyla 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 2021 animals, compared to 2020 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 covid pandemic and the online sessions in the universities.

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

There are no procedures with non-recovery severity. Mild procedures predominate.

(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 2021 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.

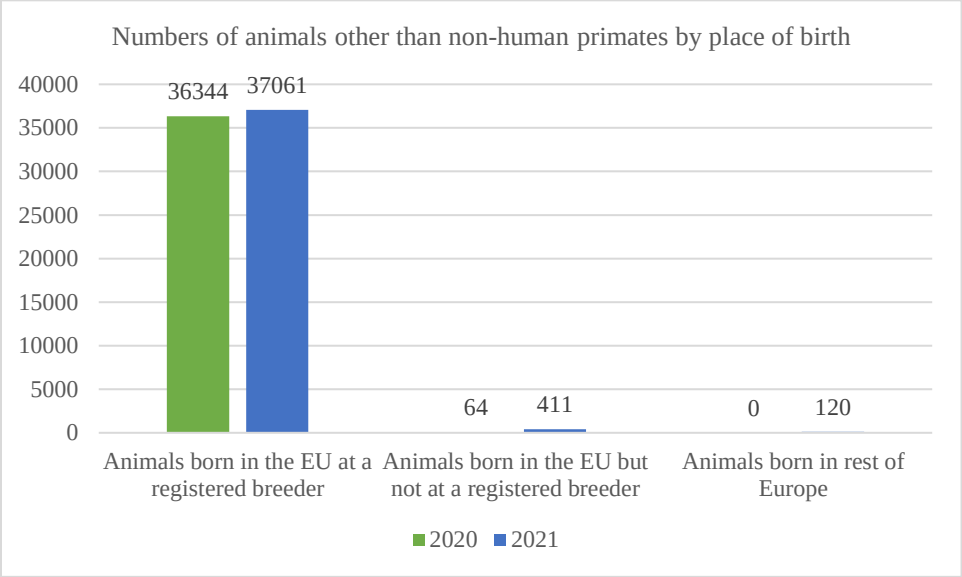
(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

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:**

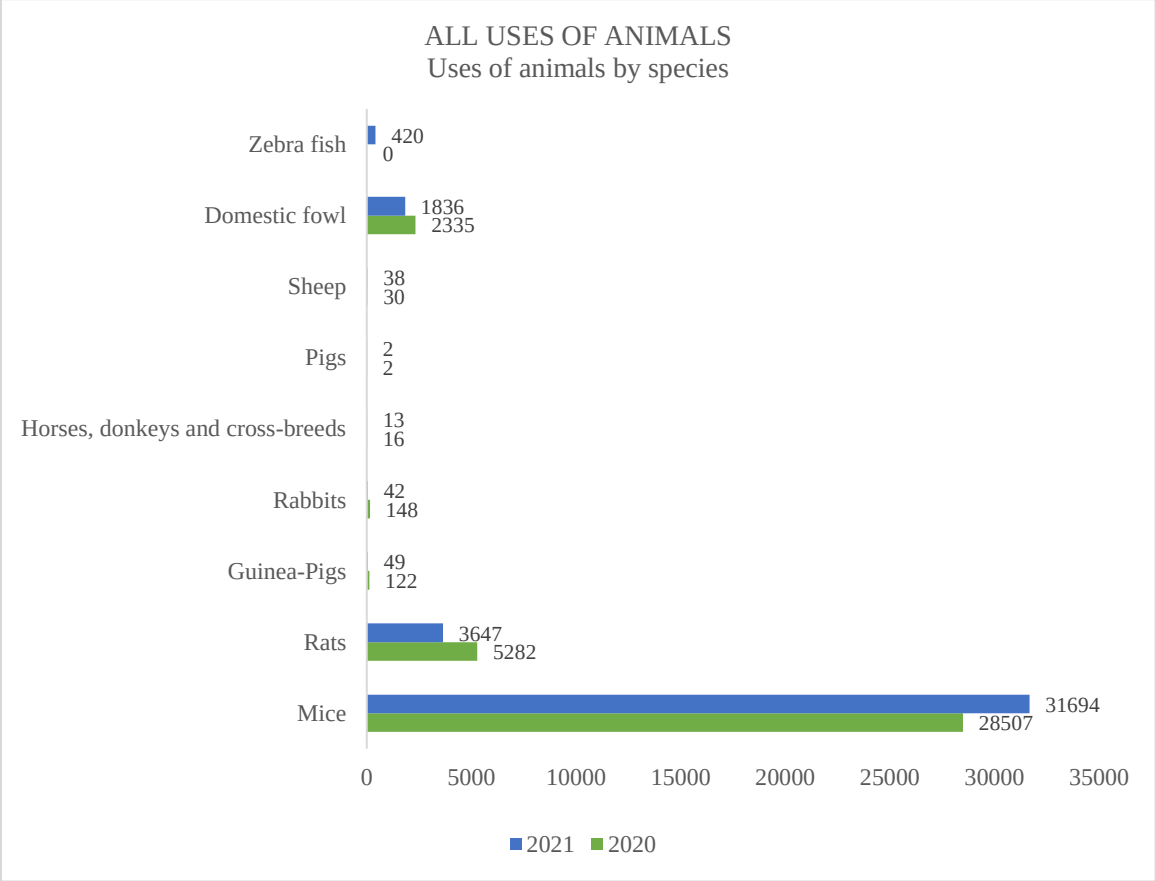
Croatia

(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 referring to increased numbers of all types of animal as follows:

- Animals born in the EU at a registered breeder increased by 717.
- Animals born in the EU but not at a registered breeder increased by 347.
- Animals born in the rest of Europe increased for 120.



The main changes observed in relation to the use of animals per species between 2020 and 2021 were:

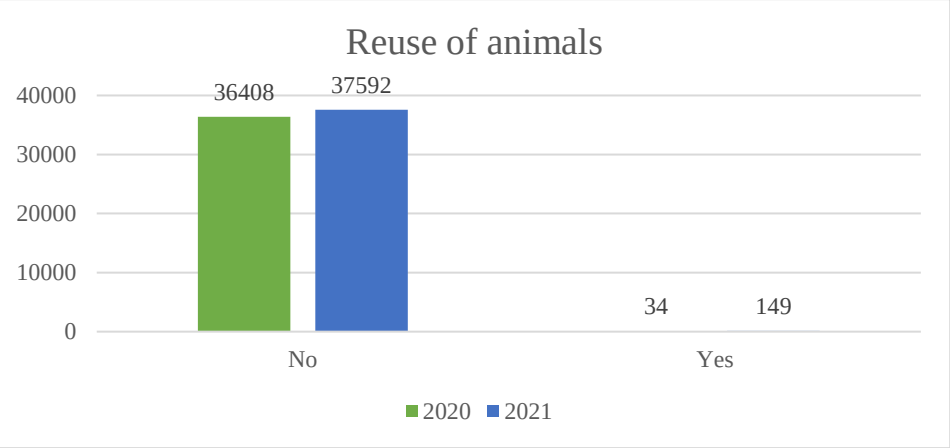
- Increase in the use of mice (+3,187) and Zebra fish (+420).
- Decrease in the use of rats (-1,635), Guinea-Pigs (-73), rabbits (-106), domestic fowl (-499), and horses, donkeys, and cross-breeds (-3).
- Sheep saw a slight increase (+8).
- The use of pigs remained unchanged.

When categorize the total use of animals at cold-blooded vertebrates and warm-blooded vertebrates, the following changes were observed between 2020 and 2021:

- An increase of 420 Cold-blooded vertebrates (fishes)
- An increase of 879 Warm-blooded vertebrates (birds and mammals).

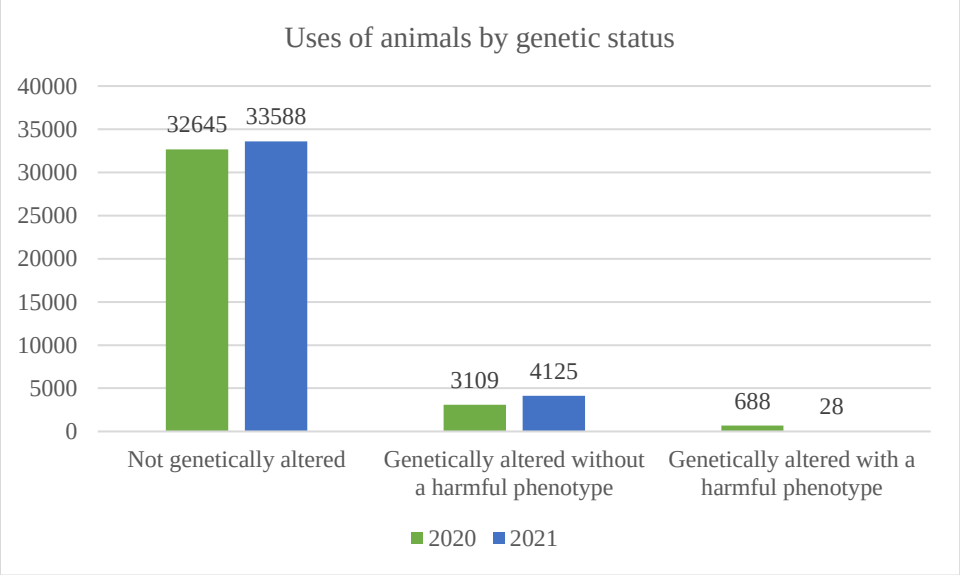
Regarding the categorization of animal species, mammals were the most common species used for scientific purposes. According to that there was an overall increase of 1,378 in the uses of animals between 2020 and 2021.

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 2021 an increase of 1,184 animals that were not reused in scientific purposes was observed, but also an increase of 115 animals that were reused for the aforementioned purposes.

Out of a total of 37,741 animals used in 2021, 37,592 were used for the first time, and 149 of them were re-used again.

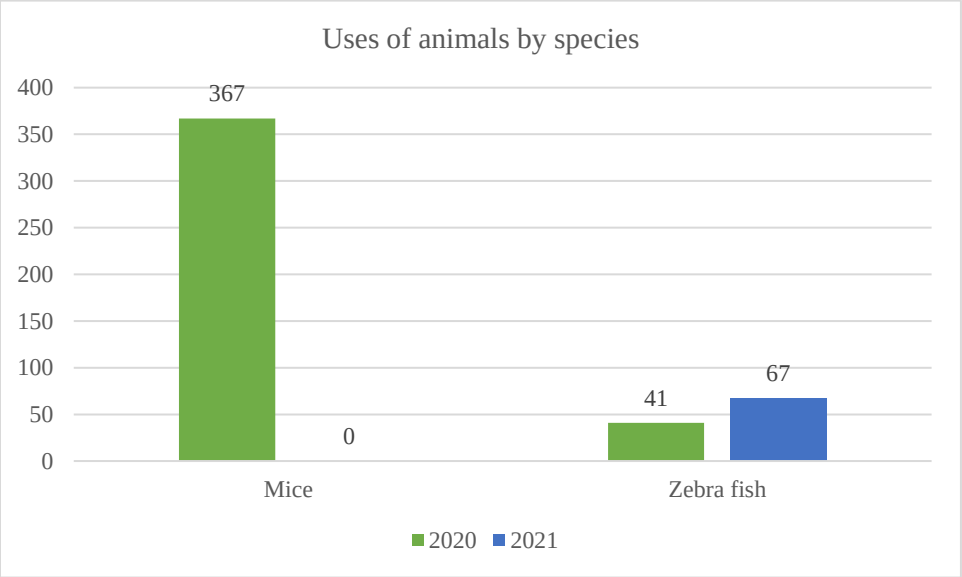


Regarding difference in the genetic status of animals between 2020 and 2021the following was observed:

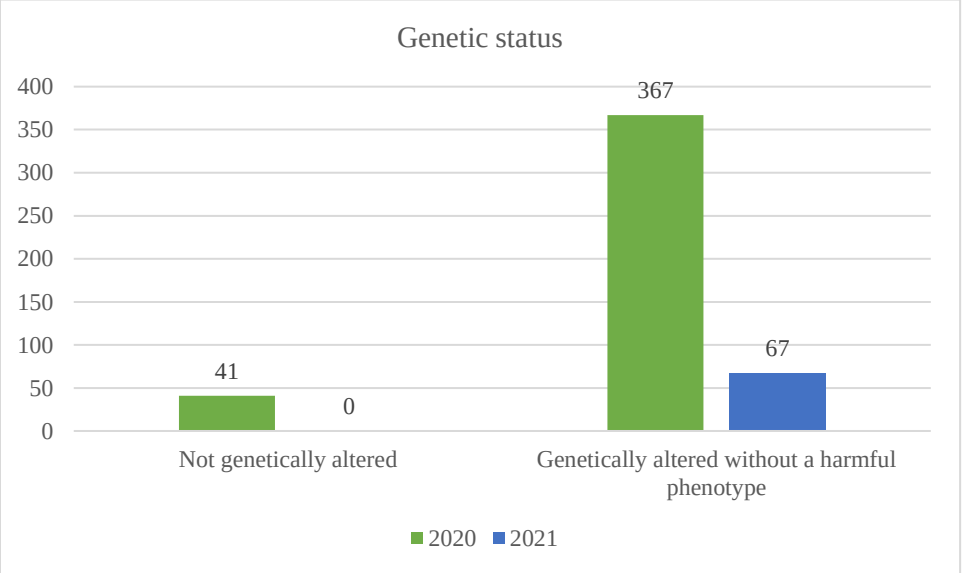
- No of not genetically altered animals increased by 943 animals
- No of genetically altered animals without a harmful phenotype also increased by 1,016
- No of genetically altered animals with a harmful phenotype significantly decreased by 660.

When changes were summed up overall increase of 1,299 in the difference in genetic status in animals between 2020 and 2021 occured.

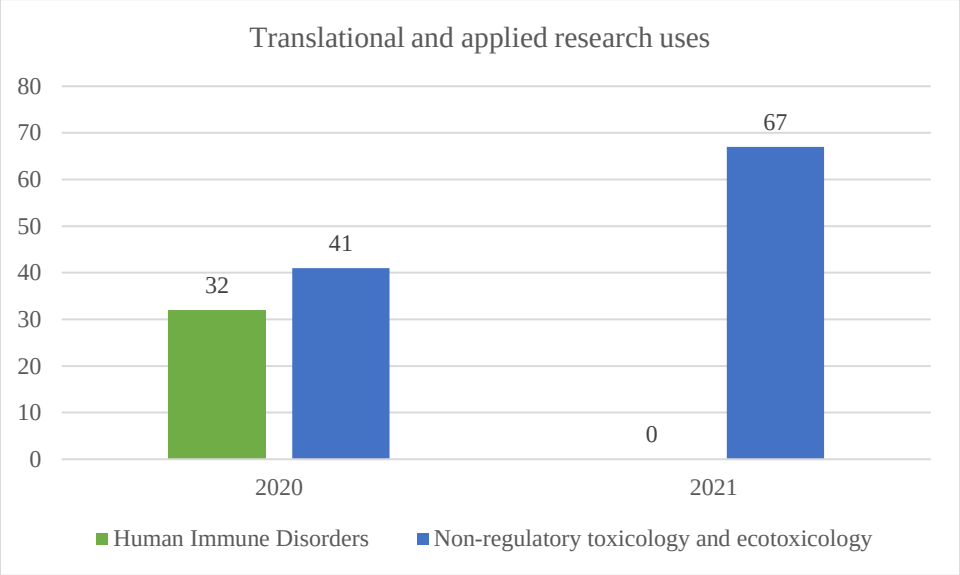
USES OF ANIMALS FOR THE CREATION OF NEW GENETICALLY ALTERED ANIMAL LINES



A significant change in the use of mice for the specified purpose was noticed between 2020 and 2021, with a decrease from 367 mice in 2020 to none in 2021. When it comes to zebra fish, 26 more were used in 2021.

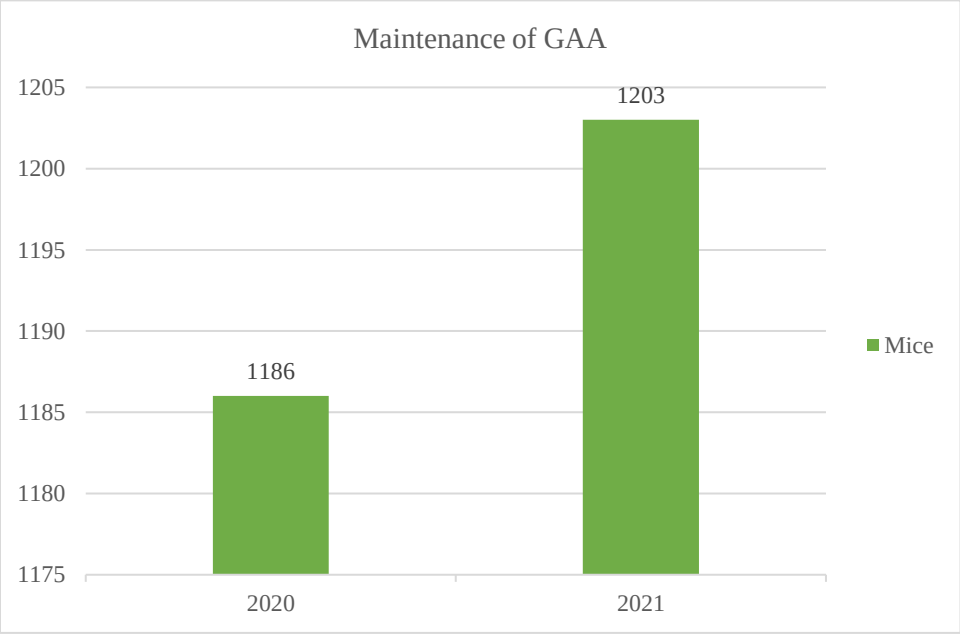


In 2021, 300 animals less than the previous year were genetically modified without a harmful phenotype, more precisely 67 of them. The same number of animals were reused in the procedures.

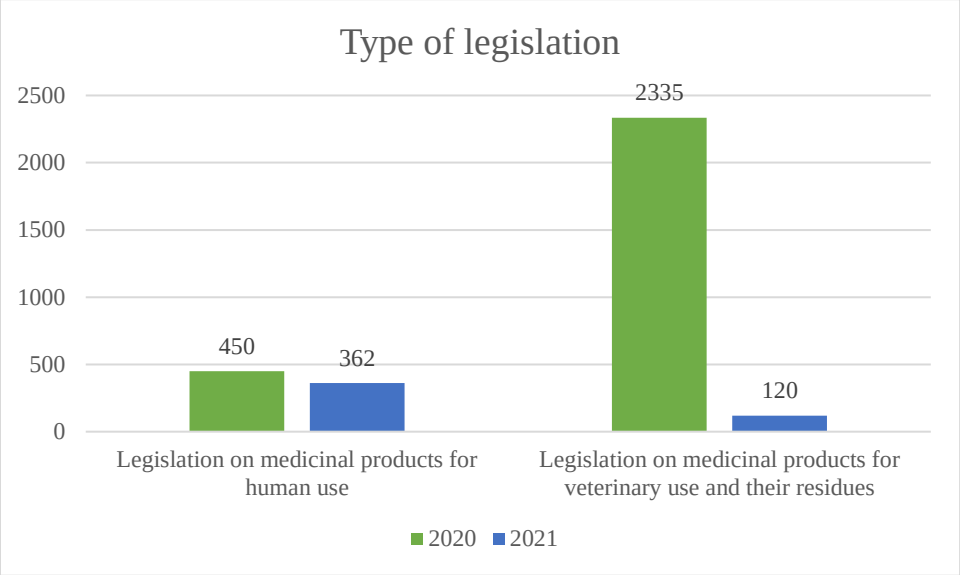


As for the uses of animals for the creation of new genetically altered animal lines, in 2021 none of the animals were used for research on human immune disorders.
For non-regulatory toxicology and ecotoxicology uses, 26 animals more were used than the previous year.

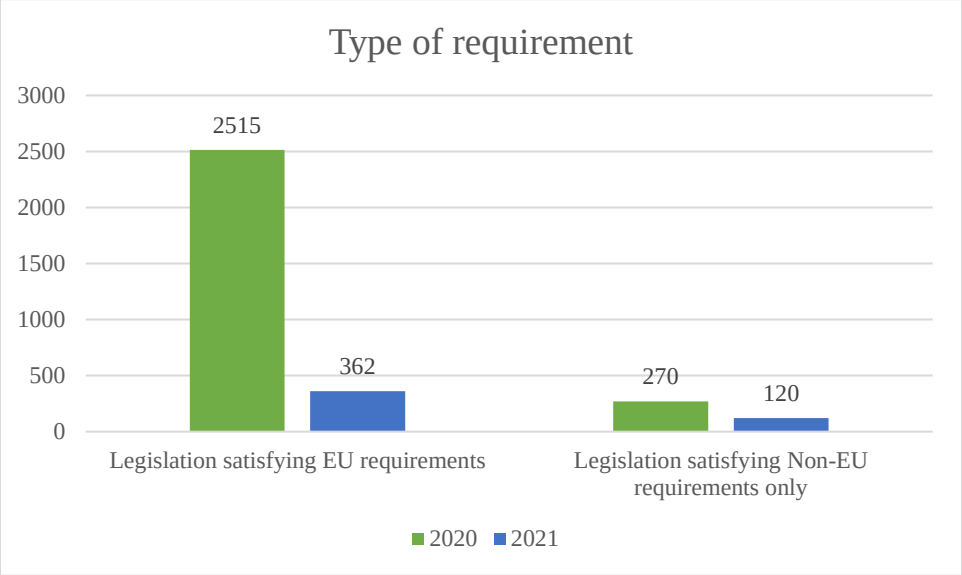
USES OF ANIMALS FOR THE MAINTENANCE OF GENETICALLY ALTERED ANIMAL LINES



17 mice more were used in 2021 for the maintenance of GAA. Also, all 1203 mice were used for the first time and all were genetically altered without a harmful phenotype.

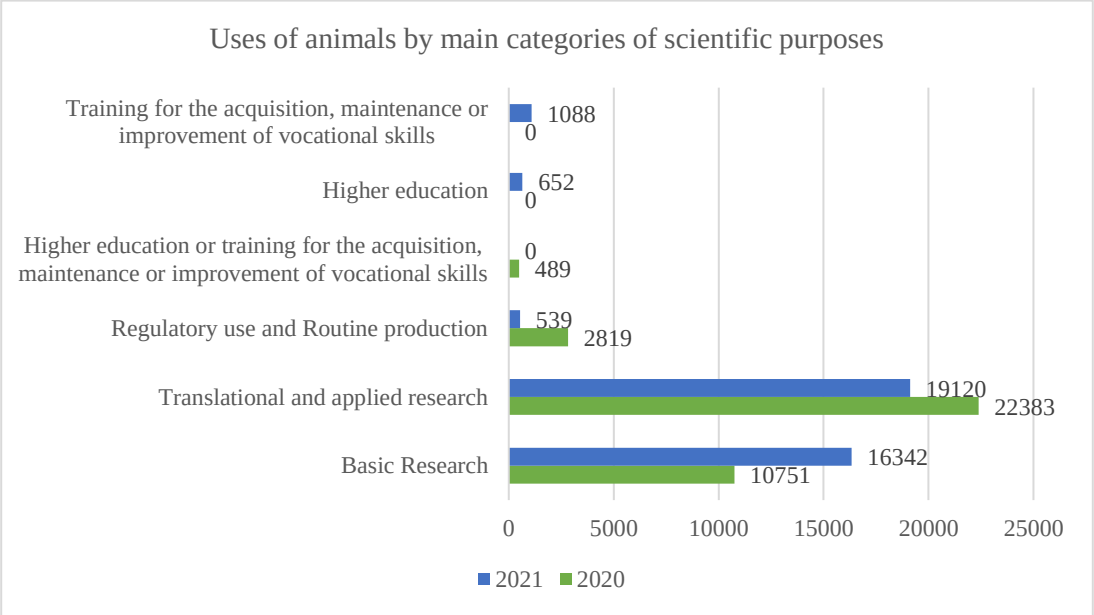


Between 2020 and 2021 there was a slight drop when it comes to legislative actions related to medicinal product for human use (by 88), and there was significant decrease in legislative actions related to medicinal products for veterinary use and their residues (by 2,215).



In 2021, number of legislative satisfying EU requirements decreased significantly (by 2,153). Legislation satisfying Non-EU requirements only also decreased, albeit to a lesser extent (by 150).

(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:

- an increase of 5,591 animals used for basic research between 2020 and 2021.

Translational and applied research:

- a decrease of 3,263 animals used for translational and applied research between 2020 and 2021.

Regulatory use and Routine production:

- a decrease of 2,280 animals used for regulatory use and routine production between 2020 and 2021.

Higher education or training for the acquisition, maintenance, or improvement of vocational skills:

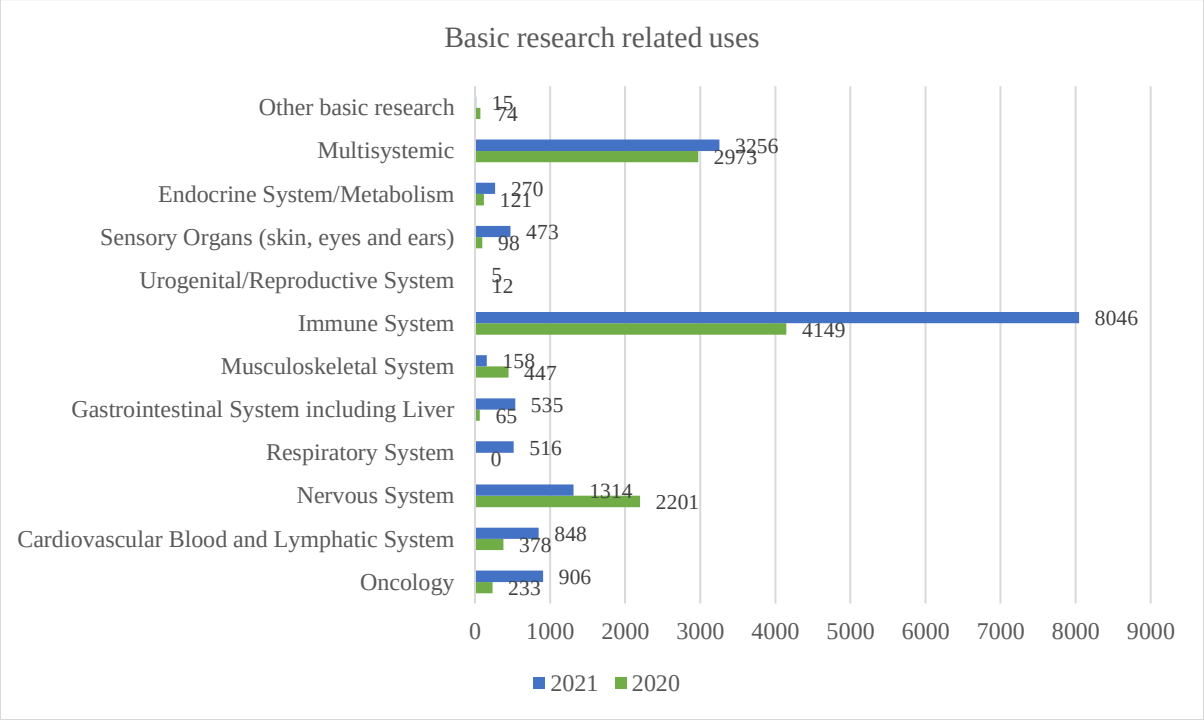
- a decrease of 489 animals used for higher education or training between 2020 and 2021.

Higher education:

- the introduction of 652 animals used for higher education between 2020 and 2021.

Training for the acquisition, maintenance, or improvement of vocational skills:

- the introduction of 1,088 animals used for training between 2020 and 2021.



Oncology:

- an increase of 673 animals used for oncology research between 2020 and 2021.

Cardiovascular Blood and Lymphatic System:

- an increase of 470 animals used for cardiovascular blood and lymphatic system research between 2020 and 2021.

Nervous System:

- a decrease of 887 animals used for nervous system research between 2020 and 2021.

Respiratory System:

- the introduction of 516 animals used for respiratory system research in 2021.

Gastrointestinal System including Liver:

- an increase of 470 animals used for gastrointestinal system research between 2020 and 2021.

Musculoskeletal System:

- a decrease of 289 animals used for musculoskeletal system research between 2020 and 2021.

Immune System:

- an increase of 3,897 animals used for immune system research between 2020 and 2021.

Urogenital/Reproductive System:

- a decrease of 7 animals used for urogenital/reproductive system research between 2020 and 2021.

Sensory Organs (skin, eyes, and ears):

- an increase of 375 animals used for sensory organs research between 2020 and 2021.

Endocrine System/Metabolism:

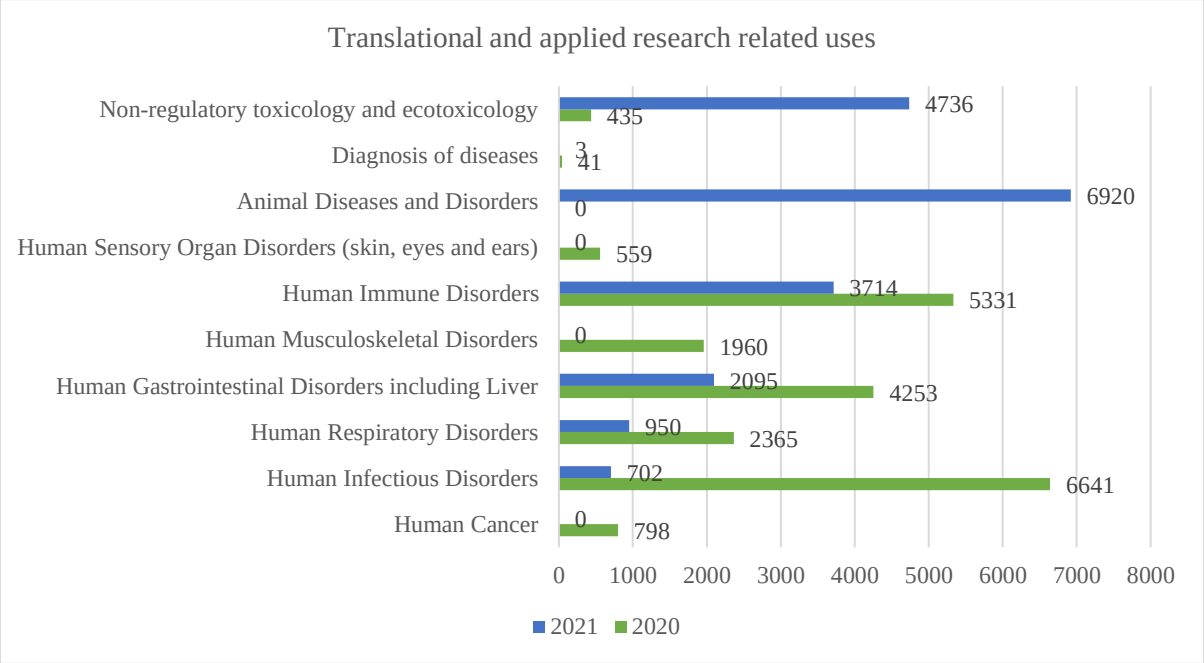
- an increase of 149 animals used for endocrine system/metabolism research between 2020 and 2021.

Multisystemic:

- an increase of 283 animals used for multisystemic research between 2020 and 2021.

Other basic research:

- a decrease of 59 animals used for other basic research purposes between 2020 and 2021.



Human Cancer:

- a decrease of 798 animals used for human cancer research between 2020 and 2021.

Human Infectious Disorders:

- a decrease of 5,939 animals used for research on human infectious disorders between 2020 and 2021.

Human Respiratory Disorders:

- a decrease of 1,415 animals used for research on human respiratory disorders between 2020 and 2021.

Human Gastrointestinal Disorders including Liver:

- a decrease of 2,158 animals used for research on human gastrointestinal disorders including liver between 2020 and 2021.

Human Musculoskeletal Disorders:

- a decrease of 1,960 animals used for research on human musculoskeletal disorders between 2020 and 2021.

Human Immune Disorders:

- a decrease of 1,617 animals used for research on human immune disorders between 2020 and 2021.

Human Sensory Organ Disorders (skin, eyes, and ears):

- a decrease of 559 animals used for research on human sensory organ disorders between 2020 and 2021.

Animal Diseases and Disorders:

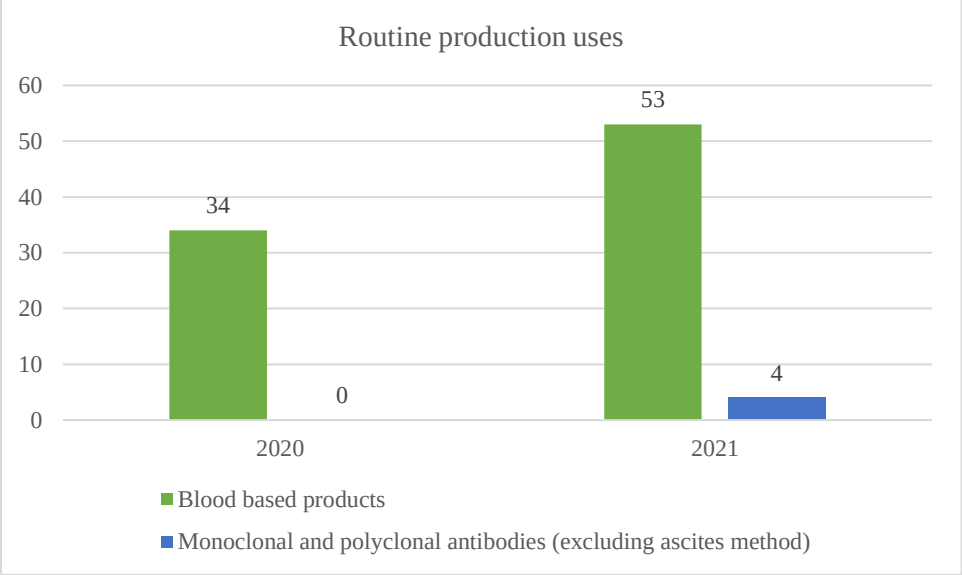
- the introduction of 6,920 animals used for research on animal diseases and disorders between 2020 and 2021.

Diagnosis of Diseases:

- a decrease of 38 animals used for the diagnosis of diseases between 2020 and 2021.

Non-regulatory toxicology and ecotoxicology:

- an increase of 4,301 animals used for non-regulatory toxicology and ecotoxicology research between 2020 and 2021.

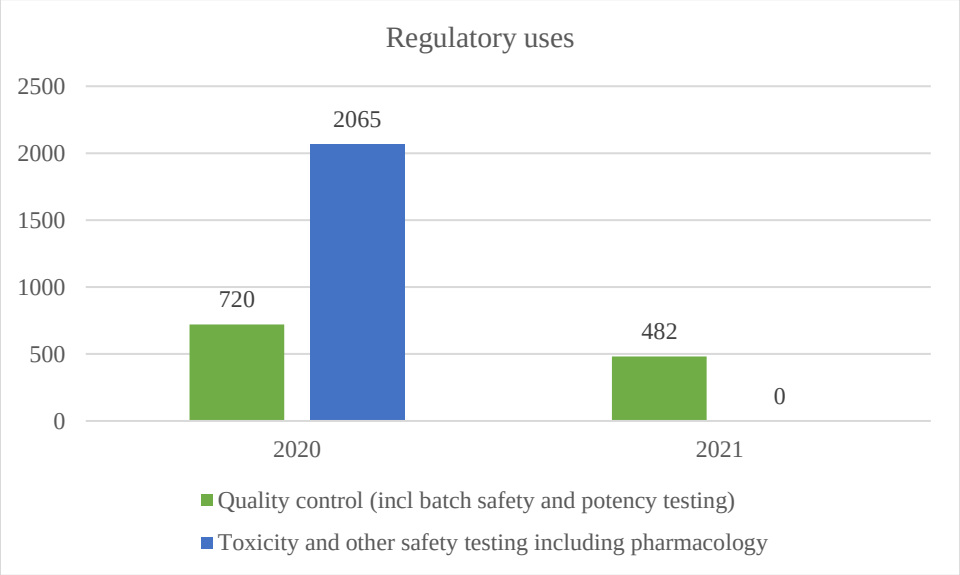


Blood-based products:

- an increase of 19 animals used for the production of blood-based products between 2020 and 2021.

Monoclonal and polyclonal antibodies (excluding ascites method):

- the introduction of 4 animals used for the production of monoclonal and polyclonal antibodies (excluding ascites method) between 2020 and 2021.

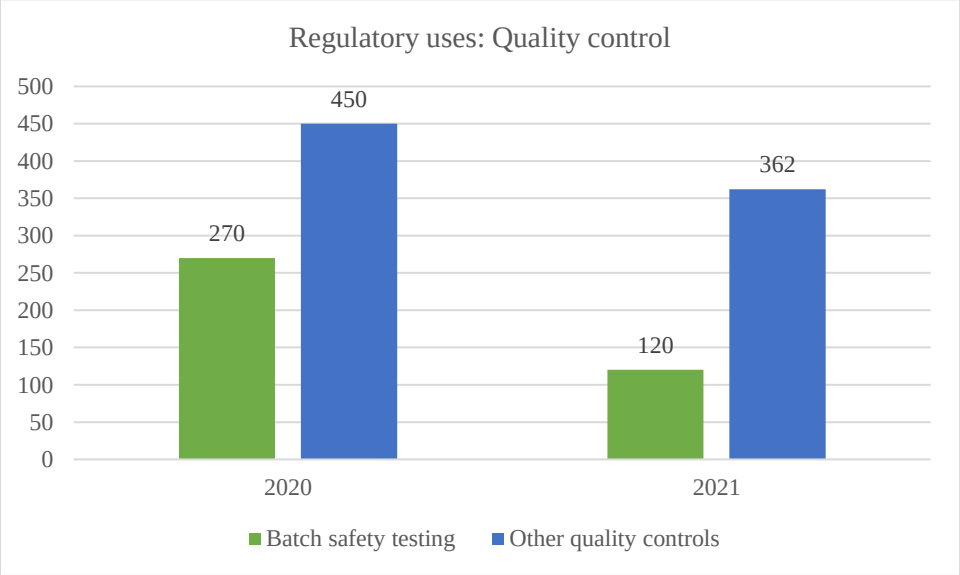


Quality control (including batch safety and potency testing):

- a decrease of 238 animals used for quality control purposes between 2020 and 2021.

Toxicity and other safety testing including pharmacology:

- a decrease of 2,065 animals used for toxicity and other safety testing between 2020 and 2021.

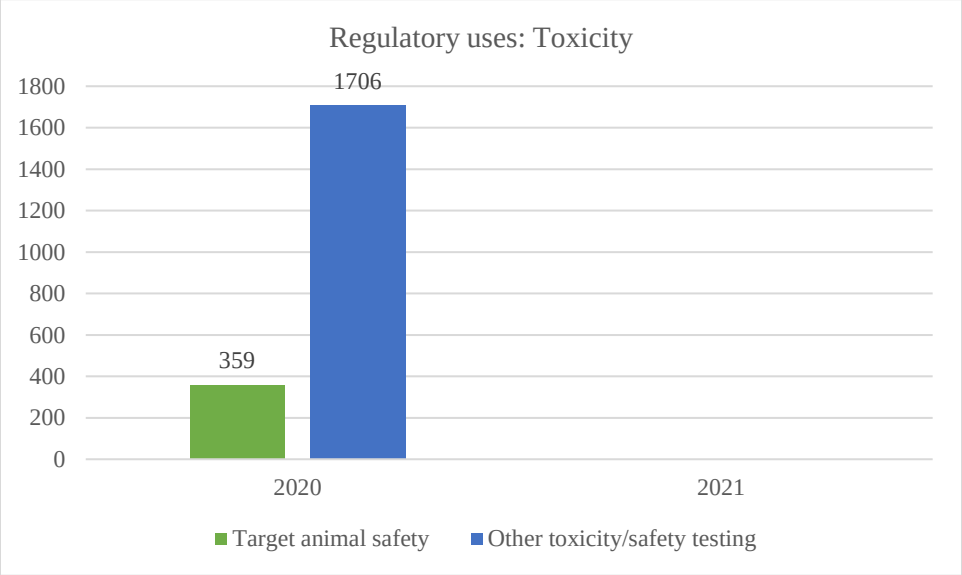


Batch safety testing:

- a decrease of 150 animals used for batch safety testing between 2020 and 2021.

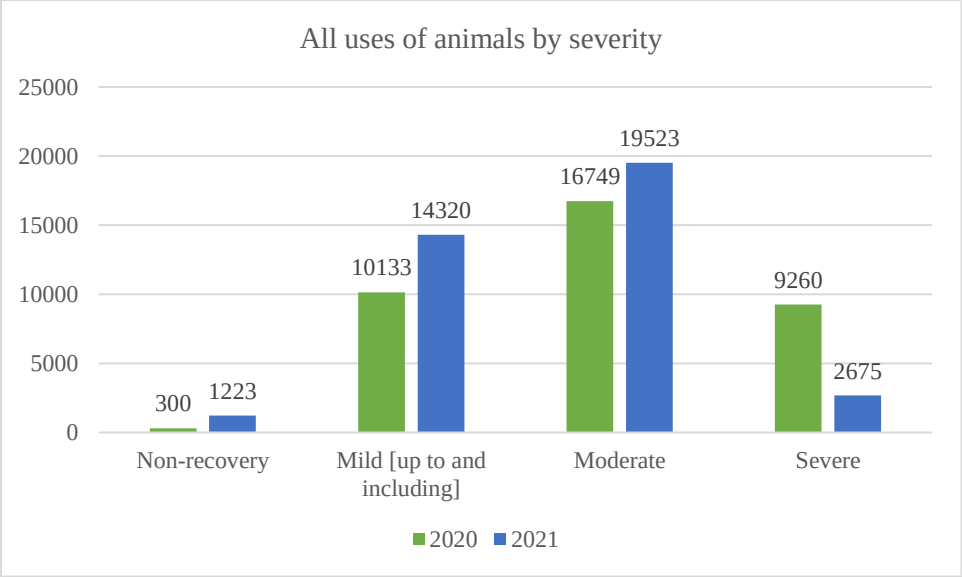
Other quality controls:

- a decrease of 88 animals used for other quality control purposes between 2020 and 2021.



There is no data provided for 2021 on target animal safety and other toxicity/safety testing.

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



Non-recovery procedures:

- in 2021, this number increased by 923 animals

Mild [up to and including]:

- in 2021, this number increased by 4,187 animals

Moderate:

- in 2021, this number increased by 2,774 animals

Severe:

- in 2021, this number decreased by 6,585 animals

(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" a few animals (15) were used for the purpose of environmental impact on genome mutation.
In category "Other quality controls" for the purpose of stability testing and method validation, in accordance with regulations on medicinal products for human use, 362 mice were used to test the stability of an antitoxin used for the treatment of humans bitten by poisonous snakes.

(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:

Generally, there were no significally changes observed in comparison to 2020. In 2021 the number of projects where animals were used was 29 whilst in 2020 was 30. Comparing to 2020, the number of animals used in 2021 fell slightly. As in previous years, the greater proportion of animals used in 2021, concerns mice. Also a small number of rabbits and frogs were used. In addition, a small amount of cats and dogs were used in procedures. Zebra fish were not used in 2021 since the project in which they were used was expired.

(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 slight fall in the number of animals used under severe severity procedures is observed, comparing to 2020 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:

In 2021, the proportion of animals used in basic research and reported under the category "other", was not significant.

(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 2021.

(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 were no such cases for the year 2021.

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 increased between 2020 and 2021. Opposite to this numbers of animals used in category *"Preservation of species"* started decreased and number of animals is like number in 2019. There were not used any NHP in 2021. There is decrease in numbers Re-Use animals. There is 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:

Overall numbers increased between 2020 and 2021. Increase is especially in category *"Basic Research", "Translational and applied research"* and "*Protection of the natural environment in the interests of the health or welfare of human beings or animals"*. Perhaps due to COVID pandemic and lockdown in 2020 there was significant decrease between 2019 and 2020. Number of uses of animals in 2021 is similar to 2019.

Category *"Higher education or training for the acquisition, maintenance or improvement of vocational skills"* was divided in to "*Higher education"* and "*Training for the acquisition, maintenance or improvement of vocational skills"* in 2021. Total number of animals used in education and training was increased.

There is significant increase in category *"Legislation satisfying EU requirements"* and opposite to this decrease in category "*Legislation satisfying national requirements only [within EU]*".

Opposite to 2020 there is increase in the use of animals by type of legislation in category "*Legislation on medicinal products for veterinary use and their residues*", "*Legislation on medicinal products for human use*" and "*Industrial chemicals legislation*".

There is significant decrease of *"Other fish"* category due to new category *"Guppy, swordtail, molly, platy"* and *"Salmon, trout, chars and graylings"*.

Opposite to previous years there were use less rabbits in "*Regulatory use and Routine production*". Experiments were for production of antigen of Rabbit haemorrhagic disease.

There is 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 continual increasing of moderate uses. Opposite to this there is decrease of severe uses in 2021 caused by decrease numbers of fish experiments regarding waste legislation and toxicity of

waste. There is also increase of mild uses between 2020 and 2021. There are continual decrees of non-recovery uses.

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

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 "*Other Fish*", more than 25 % are of specie *Ancistrus* sp. used for the purpose "*Translational and applied research Animal Nutrition*", more than *19 %* of uses was *Cyprinus carpio* and 10 % of uses was *Perca fluviatilis*. There are mainly experiments of university who deals with breed fishes.

In category "*Other birds*" more than 34% are of specie *Hirundo rustica* and 20 % *Passeriformes.* The purpose of uses was "*Basic research Ethology/Animal Behaviour/Animal Biology*".
In category "*Testing by Legislation*", "*Other Legislation*" included mainly experiments for the purposes of waste legislation and toxicity of waste.

Category "*Other Basic Research*" mainly included experiments on parasitology with different species.

In "*Regulatory use and routine production - Routine production*" in "*Other product types*", 81,55 % of experiments were for purposes of acquisition of live attenuated lines of selected species of chicken coccidian for vaccine production and 17,4 % of experiments were for production of antigen of Rabbit haemorrhagic disease.

(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 75 mouses use for production of monoclonal antibodies by mouse ascites method and 12 rabbits were use 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 2021.

Denmark

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

In 2021, the overall number of animals used for experimental procedures in Denmark was 220,611. The number is lower than in 2020, where the total number of animals was 259,411 resulting in a 15 % decrease.

The large decrease in numbers is due to the fact that a research project on diseases in rainbow trout has expired in 2020. In a small country like Denmark, a changed focus from a few research groups can have a large impact on the statistics.

The majority of experimental procedures used mice (65 %), rats (11 %) and pigs (8 %). Together these species were used in approx. 84 % of all experimental procedures in 2021. A high percentage of experimental procedures involving mice and rats were used for Human Endocrine / Metabolism Disorders, Human Cancer and Human Nervous and Mental Disorders (translational and applied research purposes). Pigs are mainly used for experimental procedures involving Animal welfare (translational and applied research purposes).

The overall distribution in purposes of procedures for all animal species are 36 % Basic research, 54 % Translational and applied research and 7 % Regulatory testing for 2021.

The severity assessment for 2021 shows that 49 % of experimental procedures in animals were mild and 46 % were moderate. Only about 1,7 % of the animals used for experimental procedures experienced severe suffering in 2021 and this confirms the trend from the previous years.

(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 other carnivores is zero in 2021 due to the actions taken towards COVID-19 infections in mink, which resulted in euthanasia of all mink in Denmark in 2020. Due to an increased focus on animal welfare in conventional farming the number of pigs and cattle are slightly higher in 2021. For Rana the number is also zero because the only research group using this species haven't been active in 2021.

For the category other fish (including Sea bass and Salmon, trout etc.), there has been a large decrease in the total number. This is due to the fact that a research project on diseases in rainbow trout has expired in 2020 as mentioned above. We expect the numbers to increase again in 2022.

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

Compared to the number from previous years, the numbers are relatively stable and consistently low. As Denmark generally has few animals experiencing severe severity, a changed focus from just one or two research groups can affect the statistical outcome in one way or the other.

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

The Danish National Committee supports the animal welfare bodies by hosting a yearly meeting, providing platforms for sharing best practice and dissemination of guidelines.

An updated website for the National Committee has been launched, having a strong focus on disseminating best practice, primarily towards animal technicians and animal caretakers.

The Danish 3R-center is still working hard to promote the 3R's and one way is by funding research. Another event is the 3R-center's annual symposium, which is open to all interested. Further information is available on www.3rcenter.dk.

The Danish Animal Experiments Inspectorate hosts three annual mini-seminars for both scientific staff and animal caretakers. The seminars provide great opportunities for discussing best practice and new models, as well as disseminating information on the legislation and correct statistical reporting.

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

In three cases the use of the category "other" is relatively high in Denmark – this concern "other birds", "other fish" and "other amphibians".

In 2021, some research groups have focused on research within disease monitoring of wild animals, hence the number of different bird species and fish. One research group began research into regenerative properties using Axolotl.

(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 registered cases of these categories.

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

In Denmark, the "severe" classification was not exceeded in any cases in 2021.

Estonia

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

3886 animals were used for scientific purposes in 2021. Compared to 2020 (4089 animals), there has been a slight decrease in the use of animals. No animals were reused in 2021.

All animals used in 2021 were born in the EU. Of them 51.31% at a registered breeder (63.22% in 2020), and 48.69% not at a registered breeder (36.78% in 2020). In 2021, use of animals by genetic status is similar to previous year - 84.77% of all animals used in 2021 were not genetically altered and the remaining 15.23% of animals were genetically altered, but without a harmful phenotype. In 2020 82.78% were not genetically altered and 17.22% GA animals without a harmful phenotype.

In 2020 all animals used were warm-blooded vertebrates, in 2021 ⅔ are warm-blooded and ⅓ cold-blooded vertebrates. All cold-blooded vertebrates used in 2021 were fish: 81 salmon, 1185 other fish (Eurasian perch and European flounder). Of warm-blooded vertebrates, 2244 (~56% overall) were mammals and 376 (~10 % overall) birds. The most used species in 2021 was mice with a total number of 1563 accounting for 40.22% of all uses (1864 mice for 45.59% in 2020). Use of rats has decreased from 669 in 2020 to 375 in 2021, also a significant decrease in use of cattle – 1425 animals in 2020 compared to 254 in 2021. 302 other birds were used in 2021, but none in 2020.

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

No significant changes in uses of animals in regulatory use and routine production, in 2020, 131 animals were used, in 2021, 126 animals. Same for training for the acquisition, maintenance or improvement of vocational skills - in 2020, no animals were used, but in 2021, 4 animals (0.1% of overall uses).

In previous years, no animals were used for the protection of the natural environment in the interests of the health or welfare of human beings or animals. In 2021, 539 animals (13.87% overall) were used and this increase is connected to a project authorised in 2021 for this purpose.

Use of animals decreased in translational and applied research from 1521 uses in 2020 (37.2% of all uses) to 481 in 2021 (12.38% overall). A study for the purpose of researching animal diseases and disorders, was conducted in previous years, which increased the numbers of animals used for translational and applied research significantly for 2020 (most of the animals were used for this study). No such large-scale project was carried out in 2021.

2736 animals (2437 in 2020) were used in basic research with an increase in use of animals in “other” basic research (613 uses accounting for 15.77% overall in 2021, none in previous years). The reason for the increase in the rise of animals used in basic research can be attributed to a higher number of project authorisations granted in 2020 and 2021.

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

Of all uses in 2020, 63.85% were classified as mild, 28.49% moderate, 0.86% severe and 6.8% non-recovery. In 2021, 52.8% were classified as mild, 41.77% moderate, 2.42% severe and 3.01% non-recovery.

Projects, where ~81% of all uses of animals were reported for 2021, were authorised in 2020 and 2021. The increase in moderate and severe and decrease in mild severities correlates generally with projects authorised in these years, the estimated severities of procedures and the numbers of animals authorised to be used in the projects.

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

The ethics committee evaluates each project application thoroughly to make sure that 3Rs have been considered and applied accordingly before authorisation.

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

The proportion of animals classified as "other" was 38.26%. In total 1487 animals, of which 1185 fish and 302 birds.

Category "other birds" included 15 great tits (*Parus major*), 2 corn crakes (*Crex crex*) and 285 pied flycatchers (*Ficedula hypoleuca*). All of these animals were used for basic research purposes.

Category "other fish" included 613 Eurasian perch (*Perca fluviatilis*), 539 European flounder (*Platichthys flesus*) and 33 pikeperch (*Sander lucioperca*). 539 of them were used for the purpose of protection of the natural environment in the interests of the health or welfare of human beings or animals. 646 were used for basic research purposes, 613 of them for other basic research, namely for researching adaptation in 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:

Animals have been used for the purpose of routine production of monoclonal and polyclonal antibodies, excluding ascites method. 126 animals (74 domestic fowl, 4 mice, 48 rabbits) were used in 2021 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

There were no cases where severity exceeded the “severe” classification.

Finland

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

The overall picture changed little from previous years. Procedures (with GM creation and maintenance) totalled 104 886 (compared to 119 986 in 2020). The changes in individual research areas can be considered as normal annual variation in animal research. However, the use of rats has decreased over the past five years (from approximately 15 000 to 10 000 animals).

There was an exceptional increase in the number of procedures involving the creation of GM mice (from 6 915 in 2020 to 12 082 in 2021). This was due to active work by one of the GM service units.

The number of procedures with dogs and cats remained at previous years' levels due to a research project in which disease genes were studied (141 cats and 1 086 dogs). In this project, single blood samples were taken from pet animals.

(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 decrease in animal use in both basic (–11 394 procedures) and translational research (–9 487 procedures), the two most important areas of research in Finland. No special reasons for the changes were determined. However, there was a reduction in procedures in both basic (–1 628 procedures) and translational (–3 042 procedures) neurological research.

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

Severe procedures (4 687) remained at 2020 levels. 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.

(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 was established: https://fin3r.fi/en. The Centre organizes education and training on 3R issues for researchers.

(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 'other' categories, a total of 7 889 fish (Salmonidae) were used for research on evolutionary genetics.

(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 and sheep for diagnostic purposes, altogether 465 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 2021 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 617 in 2020 to 589. Of these 589 establishments, 36 stated that they had no uses to report for 2021.

The total number of uses of animals declared in 2021 was **1 893 897**, as compared to 1 643 787 in 2020 and 1 865 403 in 2019. The steady downward trend in the number of uses observed since 2016 is broken this year. The unrepresentative nature of the number of uses in 2020, the year of the pandemic, was highlighted in the previous report, which anticipated an increase in 2021. The fact that the number of uses for 2021 is higher than in 2019 could be partly explained by a 'rebound' effect linked to the resumption of research projects discontinued or suspended during the lockdown periods in 2020. The number of uses in 2021 also includes new research projects directly linked to the fight against COVID-19.

For a better analysis, the data for 2021 will be compared to that for 2020 as well as that for 2019, which is representative of research activity prior to the pandemic.

Species used

The figures for 2021 are broadly similar to those for 2020 and 2019 and attest to the dominance of the mouse model in experimental procedures (**61%** of uses). Rabbits (**9.1%**) and rats (**8.7%**) remain the two next most popular species. Fish account for **10.5%** of uses, all species combined.

The number of uses of cats remains stable at **1 018**, compared to 970 uses in 2020 and 1 007 in 2019. These uses are mainly regulatory tolerance tests for veterinary medicines and nutritional studies. Of these uses, **90%** are classified as 'mild' (78% in 2020 and 71% in 2019).

Uses of dogs increased compared to 2020 but decreased compared to 2019, with **4 383** uses (4 079 in 2020 and 4 898 in 2019). This is a fall of **11%** on 2019. The proportion of dogs being re-used was **41%** (identical to 2020 and compared to 37% in 2019). The tests carried out mainly concern regulatory toxicology in human or veterinary medicine (**93%**). Of these uses, **68%** are classified as mild (66% in 2020, 54% in 2019).

The use of primates decreased from 3 996 to **3 593**, a fall of 11% on 2020, when there was a significant increase in activity connected in particular to the COVID-19 pandemic. The number of uses of primates reported in 2021 is close to that of 2019 (3 339), compared to which there has been an increase of 8%.

Primates used for the first time are from parents born in captivity in **76%** of cases (F2 generation; 68% in 2020, 66% in 2019). As of this year, the criteria have been amended to better identify F2 generation primates and primates from colonies not containing wild-caught animals (self-sustaining colonies). They constitute 80% of cases; the remaining 20% being first-generation captive-born animals. The proportion of primates being re-used was **21.5%**, compared to 44% in 2020 and 38%

in 2019. The explanation for this decrease in the rate of re-use of primates is currently not known. If the trend continues in future years, it will be further investigated.

49.7% of the uses of primates relate to legislation on medicinal products for human use.

Salmonids such as salmon (50 187 uses) and moronidae such as sea bass (16 912 uses) – two new categories in the 2021 statistical survey – account for 34% of uses of fish. These two categories plus the 'other fish' category (64 428 uses) correspond to an increase of 55% on 2020 and a decrease of 26% on 2019. The increase is largely due to an increase in the use of salmonids and moronidae.

Uses of birds other than hens increased by 13% following a 44% decrease in 2020. This category includes many different species, including ducks and geese as well as wild bird species (pheasants, white-throated dippers, zebra finches, etc.) for which the research fields (ethology, disease studies, conservation) often include field studies. These fields were particularly affected by the travel restrictions in 2020. Compared to 2019, the number of animals decreased by 37%.

The turkey (*Meleagris*) is a new category with 13 64 [*sic*] uses, of which 11 781 were related to the production of vaccines for poultry.

Species that have seen an increase in uses include the golden hamster with **12 224** uses (+ 3 757 compared to 2020 and + 6 312 compared to 2019) and the ferret with 251 uses (+ 82 compared to 2020 and + 101 compared to 2019), two models of COVID-19 infection. The same is true of guinea pigs, for which the **17%** increase (34% compared to 2019) is directly linked to vaccine batch testing. The category 'other carnivores' increased from 18 uses in 2020 to **1 428** in 2021 (24 in 2019). This increase is due to the authorisation of a project to study the eco-epidemiology of bats in the context of research into emerging infectious diseases.

Re-uses

All species combined, the number of re-uses was **24 584** in 2021, compared to 34 307 in 2020 and 37 816 in 2019. This corresponds to an overall re-use rate of 1.3%. The real rate is much higher for species such as carnivores, livestock and primates, as indicated above.

Genetically altered animals

The number of uses of genetically altered animals was **26%**, as compared to 27% in 2020 and 22% in 2019. As in 2020, the vast majority of these were mice (**89%**). The proportion of phenotypes identified as harmful remained broadly stable in recent years, with **4.4%** in 2021 compared to 5.1% in 2020 and 3.3% in 2019.

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 of animal uses fall into three main areas:
- basic research;
- translational or applied research;
- regulatory uses or production of organic products for therapeutic use.

These areas together account for **92%** of uses (95% in 2020).

The share of animal uses for basic research increased from 37% in 2020 to **38% in 2021** (41% in 2019). This year again, the field of neuroscience used the most animals.

Toxicological and regulatory research for the development, production or quality and safety testing of medicines or foodstuffs follows, with **28%** of uses (31% in 2020 and 29% in 2019). In this area, there is an increase in the number of rabbit uses (+16% compared to 2020, +21% compared to 2019), which is directly linked to the production of a drug used to treat transplant rejection in humans. This drug, which is mainly produced in France, is distributed worldwide with demand growing.

Translational or applied research decreased from 27% in 2020 to **26%** in 2021 (22% in 2019), with research on cancer making up a large share of the human diseases studied. Uses in the areas of animal welfare or animal diseases concern productive animals and species for human consumption.

The following areas are much less represented but are generally increasing:

- maintaining colonies of genetically altered animals, with **3.7%** of uses (2.6% in 2020 and 3.2% in 2019);
- species conservation, with 2.6% of uses (1.2% in 2020 and 1.9% in 2019).

For species conservation, **29 960** more uses were recorded compared to 2020 (14 359 more compared to 2019). It is notable that **85%** of these uses concern fish other than zebrafish. To give some examples, a long-term monitoring project for migratory fish (salmon, trout, eel) required the use of 24 298 animals, and a restocking project for the European sturgeon – a critically endangered species – involved 10 704 animals.

The area of education and vocational training remains very stable at **1.8%** of uses.

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 (**81%**). Uses falling under the categories 'severe' and 'non-recovery' are stable, comprising **14%** and **4.6%** respectively, in line with the figures from the previous 2 years.

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'). In the context of the research framework law of 24 December 2020, France confirmed its willingness to support the development and increase the visibility of alternatives to the use of animals for scientific purposes. To this end, the law provided for the establishment of a national centre dedicated to the 'three Rs' principle, the FC3R, supported by public research, in particular at the research institute INSERM, which chairs and manages the centre.

The centre was founded in 2021 with a mission to promote the funding of research into the development of alternative methods, to develop and disseminate training on the application of the

3Rs, to promote project engineering in line with this principle, to encourage the exchange and deposition of animal lines, to make unpublished 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.

Establishing a basis in law for a structure dedicated to the specific issues of the 3Rs that is able to finance projects to develop and validate alternative methods responds to the high expectations of society and also reflects the strong engagement of academic and private research bodies in these subjects.

After a year 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 were published, one on 'The 3Rs in France: shared initiatives and sharing tools' and another on 'Replacement: alternatives to animal models and products of animal origin in research'.

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 Ethical Reflection Committee on Animal Testing, which produces recommendations, the most recent of which concerns the use and/or development of alternative methods to replace animal models in the field of antibody production (National Ethical Reflection Committee on Animal Testing).

Professional associations for animal research as well as 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 via ascites. The user establishments have been contacted individually and project authorisations are being reviewed. Restrictions on the duration of authorisations are planned, or otherwise repeals 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 'other' categories if a significant proportion of animal use is reported under this category:

In basic research, the 'other' category accounted for **0.57%** of uses, compared to 0.45% in 2020 and 0.38% in 2019. More than a third of these uses concern an embryo transfer project. Declarations in this category are subject to thorough checks in order to minimise its use.

For regulatory uses/routine production, the 'other' category accounts for **4.7%** of uses (5.2% in 2020 and 6.3% in 2019). This is due in particular to the production of toxoplasm suspension 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 84 728 uses.

As in 2019, the 'other fish' category remains sizeable, accounting for **3.4%** of uses (64 428), or **7%** including the two species not listed in 2020 (sea bass and salmon). This category includes very diverse species such as tilapia, sturgeon, carp and eel, to name but the main ones. Two thirds of the uses concern behavioural studies (21 844) and species conservation studies (20 287).

The 'other birds' category accounted for **0.5%** of uses (10 191). This category includes domestic species such as ducks or geese, as well as wild species such as pheasants, partridges and gulls. 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 **36 664** uses (compared to 40 649 in 2020), a decrease of 9.8%. This corresponds to a transition started several years ago by the Ministry of Research and the relevant user establishments.

The user establishments have all initiated efforts to develop alternative methods to the use of animals (*in vitro*) but are still encountering difficulties with differences in analytical quality, insufficient quantities, robustness of tests or time needed for validation by the competent authorities of the countries concerned. These monoclonal antibodies are employed in biomedical diagnostics and are used by many international hospitals and laboratories. Authorisations are only issued for a limited time in the interests of preserving continuity of care provision. Regular monitoring is established with the user establishments concerned. For new therapeutic products, *in vitro* tests are systematically carried out first and the use of animals is only allowed when *in vitro* testing is not conclusive.

There is also regular monitoring of existing authorisations and extensive dialogue with the user establishments concerned to support the transition to non-animal methods as soon as they are validated. This monitoring concerns in particular pyrogen testing, eye and skin irritation testing and skin sensitisation testing.

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

No authorisations were issued in 2021 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 2021, approximately 1.86 million vertebrates and cephalopods were used in Germany in animal testing within the meaning of Section 7(2) of the Animal Welfare Act (*Tierschutzgesetz*). Section 7(2) of the Animal Welfare Act defines the term 'animal test'. The figures were around 2% lower than in the previous year (2020). This continued the downward trend from previous years.

Around 81% of the animals used for testing are rodents, mainly mice and rats. Mice accounted for around 72% of the animals used and rats for around 7%. Some 12% of the animals were fish, around 3% were rabbits and around 1% were birds. Compared to previous years, 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 71% in 2020), continuing the gradual increase since 2019 (around 67% in 2019) and reaching the level recorded in 2018 (around 72%). The figure for fish used (around 9% in 2018, around 16% in 2019 and around 12% in 2020) was the same as in the previous year. The use of birds and rabbits fell in both cases by less than 1% compared to the 2020 reporting year. The figure for other animals used for testing fell to around 4%, i.e. also by 1% compared to the previous year, but was not as low as in 2019 or 2018 (around 5% in 2020, around 2% in 2019 and around 1% in 2018).

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

Killing for scientific purposes

Going beyond the requirements of the EU Laboratory Animals Directive, each year Germany also records animals killed for scientific purposes without first having undergone procedures or treatments, for instance in order to use these animals' organs or cell material for scientific purposes. Around 644 000 animals were used for this purpose in 2021, which is approximately 10 000 (around 1.6%) more than in the previous year. These animals, which were killed in accordance with Section 4(3) of the Animal Welfare Act, are not included in the number of laboratory animals notified to the European Commission.

Genetically modified animals

Despite a decrease in the overall number of animals used, the number of genetically modified animals increased further compared to previous years, with around 953 000 cases of genetically modified animals being used. The proportion of such animals was just over 51%, continuing the upward trend of recent years (around 48% in 2020 and around 43% in 2019). This concerned in particular mice (85%) and fish (13%). Notably, in 2021 only 29 rats were used to create new genetically modified lines. In 2020, the figure was over 900. At the same time, the number of genetically modified pigs, domestic chickens and amphibians has been steadily increasing since the 2018 reporting year, with the total number of such genetically modified species still accounting for considerably less than 1% of genetically modified animals in 2021. Furthermore, the number and proportion of animals used to conserve colonies of genetically modified animals but which could not be used in any further procedure notably fell by around 2% compared to 2020 (around 187 000 animals or 10% in 2020, around 157 000 animals or 8% in 2021). The distribution between species also changed. In 2020,

essentially only mice were reported in this category. However, in 2021, a significant number of zebrafish were also reported (in 2020 around 99% mice, less than 1% zebrafish; in 2021 around 88% mice and 11% zebrafish).

Primates

A further decline was observed in the number of animal tests using primates. A total of 1 886 tests using primates were reported in 2021, which is 145 fewer than in the previous year. This represents a decrease of around 7% compared to 2020, when a decline of 38% was observed compared to 2019. In 2021, a total of 1 452 primates were used for the first time, while the remaining animals (around 23%) were used again. Of the animals used for the first time, around 99% came from self-sustaining colonies, of which around 15% came from approved breeding establishments in the EU, some 34% from Africa and some 51% from Asia. Compared to previous years, the number of primates coming from self-sustaining colonies increased sharply (25% in 2018, 35% in 2019, 4% in 2020, 99% in 2021). This was primarily due to a large establishment which, until changes were made to the reporting system in 2021, had been incorrectly declaring the origin of the primates used.

As in 2020, mainly long-tailed macaques were used in 2021 (around 91% of uses in 2021 and 87% in 2020). The majority of these animals (around 96%) were used for regulatory purposes, e.g. for toxicity testing.

Dogs and cats

In the case of dogs and cats, used in particular for statutory testing, routine production of blood-based products and research on animal diseases, the number of uses, i.e. 2 657 and 862 respectively, was around the average of the last 2 years. Compared to 2020, the number of cases in which dogs were used increased by around 4% and the number of cases of cats by around 34%. However, compared to 2019, this represented a decreased of some 25% for dogs and some 10% for cats.

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 medical research. Specifically, some 56% of the animals used in animal testing within the meaning of 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 17% 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 8% 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.

Compared to 2020, the proportion of tests in the area of basic research increased by around 2%. The proportion of animals used in researching human and animal diseases also increased by around 1%. However, the proportion of animals used to maintain colonies of genetically modified laboratory animals decreased by around 2% compared to the previous year. A similar decrease of 2% was also observed in animals used for regulatory purposes and routine production.

- **Basic research**

  In 2020, as in previous years, areas of basic research included, in particular, research into the nervous system and the immune system (21% and 18%), and the cardiovascular system (around 13%). The figure for developmental biology research, which featured for the first time in 2021, accounted for just over 2% of reported animal tests in basic research. In particular, the share corresponding to 'other basic research' decreased from around 6% in 2020 to around 2% in 2021. This reduction may be due largely to efforts by the competent authorities to question, on the advice of the European Commission, any uses of the 'other basic research' category.

- **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 40% of the animals used for testing in this area were used for this purpose. The figure for animal nutrition research, which featured for the first time in 2021, accounted for just over 5% of reported animal tests in translational research.

- **Regulatory uses and routine production**

  As in previous years, there was an emphasis on batch potency testing and routine production, e.g. of antibodies, in the area of regulatory uses. In 2021, batch potency testing accounted for some 20% of regulatory uses, i.e. the same as in the previous year. The proportion of uses for routine production increased to around 21% (around 18% in 2020). Around 13% of uses could be ascribed to the production of antibodies (excluding the ascites method), a category which featured for the first time in 2021. At the same time, the share corresponding to blood-based products fell by around 9% to a total of 7% in 2021 (around 16% in 2020). Furthermore, the proportion of uses corresponding to antibody production by means of the ascites method notably decreased by 79% compared to 2020. For a total of 113 uses, the competent authorities checked whether those uses were essential in each case.

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

The severity of tests within the meaning of Section 7(2) of the Animal Welfare Act was predominantly 'mild' (approximately 63% of uses). Around 26% of the tests were classified as 'moderate' and 4% were classified as 'severe'. In 2021, the share of mild tests fell by around 4% compared to 2020 (67%) and by around 2% compared to 2019 (65%). At the same time, the share of moderate tests increased by around 2% compared to 2020 and 2019 (24% in both years). At 4%, the share of severe tests was around the average of the previous years (around 4% in 2020 and around 5% in 2019). The share of tests on animals carried out entirely under general anaesthesia and from which the animal never regained consciousness was around 6%, the same level as in 2020 and 2019. A possible reason for the partial shift from mild to moderate severity could be that the authorities have adjusted how they assess the individual severity of animal tests and that some animal tests which in recent years were classified as mild are now classified as moderate.

(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 the setting up and operation of the German Centre for the Protection of Laboratory Animals (Deutsches Zentrum zum Schutz von Versuchstieren, Bf3R) promoting research 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.

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

Category 'other animal species'

Overall, around 3% of all animals used were declared as 'other species' in 2021. The largest groups in this category were 'other mammals' (around 3%), 'other birds' (around 13%), 'other fish' (around 68%) and 'other rodents' (around 10%).

In the 'other mammals' sub-category, mainly native wild animals, in particular bats (around 62%), 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' sub-category, native wild bird species (e.g. tits, raptors and migratory birds, around 81%) and zebra finches (around 13%) 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 the 'other fish' category, primarily local wild fish (e.g. whitefish, carp, perch and European eel, around 56%) were used in basic ethological research. This may have mainly concerned fish caught in the wild that were re-released after the test was finished. The category 'salmon, trout, chars and graylings (Salmonidae)', newly included in 2021, accounted for some 11% of all reported fish. Until last year, those species were included in the 'other fish' category. However, the new category 'sea bass (spp. from families e.g. *Serranidae, Moronidae*)' had a taxonomic scope which was too narrow or, in the opinion of those carrying out the reporting, was not clearly defined. Less than 1% of fish were reported in this category and many perch-like fish continued to be included in the 'other fish' category.

In the 'other rodents' category, primarily field mice, bank voles, root voles and wood mice (around 80%) were reported. They were used in authorisation procedures for plant protection products or in basic ethological research.

Category 'other uses'

Overall, around 1% of all animal testing was reported under 'other uses' in 2021. Around 67% came under the 'other basic research' category and around 27% under the 'other regulatory purposes and routine production of other products' category.

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

- research in the field of gerontology (around 34%)
- parasitology (around 11%)
- testing of various new methods for marker, blood and biopsy sampling with the aim of refining these methods (refinement, around 35%)

The category 'developmental biology', which featured for the first time in 2021, accounted for around 2% of reported animal testing in basic research. In recent years, this testing had been included in the 'other basic research' category. Accordingly, its proportion fell to less than 2% of basic research. The sharp decrease may also be due in part to active efforts by the competent authorities to critically question the reasons for classification under 'other basic research'.

Under the category 'other regulatory purposes and routine production of other products', the emphasis was mainly on the following topics:

- monitoring of animal populations in conjunction with authorisation procedures in the area of agriculture (around 30%)
- breeding and feeding of parasites or pathogens (around 18%)

Category 'other legislation'

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

- testing of substances hazardous to water pursuant to the Administrative Regulation under 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 2021 reporting year, 40 cases were reported of pyrogenicity tests and eye or skin irritation tests under authorisation procedures or quality control for medical devices, medicinal products and chemicals. They involved 1 676 rabbits, 16 rats and 4 hamsters. 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 responses:

- in the case of 1 492 uses (around 88%) a substitute method was not or not yet recognised;
- in the case of 72 uses, it was necessary for the tests to be carried out due to standardised authorisation procedures. The following information was provided:
    - in the case of 16 uses, CPMP/ICH/286/95, February 2013,
    - in the case of 12 uses, OECD test No 402,
    - in the case of 14 uses, OECD test No 404,

    - in the case of 30 uses, the regulatory need was confirmed by the competent authority but not specified,

- in the case of 59 uses, the substitute method was not sufficiently selective,
- in the case of 73 uses, the competent authority merely reported that the use was plausible in each case.

Furthermore, in the 2021 reporting year, 113 animals were used for monoclonal antibody production by means of the ascites method. The BfR had each of these reports verified by the competent authority. In each case it received a response stating that the indicated use was correct and had a plausible justification.

(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 2021, 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 2021 by 10,64 %. 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 slightly increased use of animals born in the EU but not by a registered breeder by 5,43 % has been reported compared to 2020. This refers to the lower number of fish of domestic species (other fish) that have been used especially after the induction of sea bass as a separate species in the updated statistical template that is currently being used since 2021. This happens every year due to the fact that one establishment performs projects in fish farms that are authorised under animal health legislation, but not under the context of Directive 2010/63/EC with the derogation offered by the regional veterinary services.

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

A) A significant decrease in the number of mice used in 2021 is note compared to 2020 by 11,67%. This is attributed to the fact that no projects took place in some user establishments.

Regarding rats, their number used is also very significantly diminished to the number used in 2020 by 29,69%. This depends on the protocols authorised and completed for 2021 which did not include rats to the extent of previous years.

A significant decrease in the number of rabbits used in 2021 compared to previous years. This is due to the fact that a rabbit supply establishment has not renewed its authorisation according to the provisions of Directive 2010/63/EC. This has led to a discrepancy in the use of this species due to the shortage of animals.

A significant increase in the use of pigs is noted by 111,42 % due to an increase in higher education training programs that are performed with the use of live animals in Greece as a result of the COVID-19 pandemic. Face-to-face attendance increased in 2021.

An 25% increase in the use of domestic fowls is noted due to nutrition protocols applied by a user establishment constantly.

A significant increase in the use of zebra fish is noted due the authorisation of new user establishments dealing with this species.

B) Approximately the same proportions of not genetically altered, genetically altered mice without a harmful phenotype and genetically altered mice with a harmful phenotype is noted in Greek statistical data in 2021 compared to 2020 . Furthermore a significant increase by 25% is noted in human sensory organ studies (Translational and applied research). This is attributed to the different type of protocols performed in Greek establishments for 2021. 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 2021 again compared to other MS for 2020 but lowered by 11,25% compared to 2019. More specifically a further decrease of 50% compared to 2019 is noted. 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 production methods of aquaculture species. *Dicentrarchus labrax, Sparus aurata* and *Pagrus major* 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 2021.

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.

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 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 2020 and 2021.

J) A continuous decreased use of dogs is noted during the years, according to the relevant protocols authorised. Their number remains stable between 2020 and 2021.

(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 even further diminished by 3,32 %.

B) Animals with "moderate" severity seem to have slightly increased by 2,1%. 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 slightly variable from year to year, which can be attributed to the differeny number and nature of basic research protocols compared to 2020. Furthermore, there is a constant increase in the number of fish (gilthead sea bream and European sea bass) used with severe outcome in a user establishment. The application of experimental protocols on teleosts in this

particular user establishment had the objective to assess either resistance of fish to fish pathogens or the efficacy of vaccines under different physicochemical water parameters (i.e. different temperatures). These protocols were classified as severe because mortality is caused to 100% of fish. In 2021 all fish used for this protocol died, increasing thus the number of severely affected animals in total.

(d) Information on 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 significant proportion of other fish is constantly reported in Greece for 2021 although with an importantly decreasing trend compared to 2020. This is due to the fact that since 2021 the use of sea bass is separately reported.

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 *Argyrosomous regius* and *Seriola dumerili* 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.

(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.

(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 one user establishment in the context of a project of Translated/Applied research, Non regulatory toxicology and ecotoxicology, 3 animals were found dead. This procedure was unexpected and did not receive preauthorisation. These animals had obviously undergone unprecedented adverse effects during a toxicology testing procedure.

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 2021 was 152.709, which represents 11,83 % increase compared to 2020. The reason is for the increased use that there was more experiment in 2021, where the use of animal cannot be replaced. The proportion of re-used animals was 0.74% which 43.17 % decrease compared to 2020. The increase in the number of animals is due to vaccine testing for avian diseases on a large-scale colony (birds) and large-scale ecotoxicological testing on fish.

The number of genetically altered animal used without a harmful phenotype in 2021 was 9.226, which represent about 14,45% increase compared with the previous year. In 2021 the percentage of use is 6.04% from total uses. This also shows increase compare with 2020.

The number of genetically altered animal used with a harmful phenotype in 2021 was 584, which represent 20.10% decrease compared with 2020. In 2021 the percentage of use is 0.38% from total uses. This also shows decrease compare with 2020.

There is a significant change on the data of origin of animals compare with the previous year. In 2021 the number of animals born in the EU at a registered breeder shows 16.2 % increase, and the number of animals born in the EU but not at a registered breeder shows 49.35% decrease compare with 2020. There was not used any animal born in rest of Europe in 2021, but the used of number of animals born in rest of world shows 345.7% 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 (91.64%) of used animals were warm-blooded vertebrates. The number of fish used in experiments increased significant (from 5.666 to 12.773). The reason is a large-scale ecotoxicological testing on fish (8900 pieces of Danio rerio).

In case of guinea-pigs there was a 42.22% increase, because of a vaccine testing against animal diseases in a large number of animals. The use of hamsters also increased with 54% compare with 2020, due to virological testing.

The number of horses, donkeys and cross-breeds were increase from 17 to 58, because of experiment of musculoskeletal system with 40 animals. The use of rhesus monkey also increased from 2 to 8 in 2021, due to an experiment of human nervous and mental disorders.

The number of used sheep increased from 4 to 82 in 2012, because of two experiment of animal diseases and an experiment of musculoskeletal system.

The use of domestic fowl increased with 19.81% due to vaccine trial against avian influenza.

When analysed by the purposes of the use of animals, the following categories shows significant increase: Translational and applied research (64.72%), Higher education (from 0 to 1106), Training for the acquisition, maintenance or improvement of vocational skills (from 0 to 490). Compare with the data of 2020, the following categories shows significant decrease: Protection of the natural environment in the interests of the health or welfare of human beings or animals (64.85%), Preservation of species (from 2200 to 0), Higher education or training for the acquisition, maintenance or improvement of vocational skills (from 1007 to 0).

In the basic research related uses, the used for following purposes increased significant: Endocrine System/Metabolism (472%), Ethology / Animal Behaviour /Animal Biology (61%). The reason of the significant increase of Endocrine System/Metabolism is the environmental toxicology experiment with 1200 piece of Danio rerio.

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

The number of mild uses increased with 8%, but the proportion of mild uses was decreased from 51.67% to 50.06% and moderate uses decreased from 29.51% to 25.03%. The number of severe used shows a significant 83% increase. The reason is a large-scale ecotoxicological testing on fish (8900 pieces of Danio rerio), where the users gave the preliminary classification, not the actual classification. On the other hand, the proportions of non-recoveries decreased from 5.41% to 2.94%.

(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.

Reasons for very high animal use that, the number of animals is required and regulated by Ph. Eur in force. Animals are used in quality control batch release tests (e.g.: 15080 Gallus gallus domesticus).

(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 8.88% from the total 42859. These were mainly Duck Cherry Valley hybrid (54.57%), Pekin Duck (10.25%), Muscovy Duck (8.86%), Zebra Finch (Taeniopygia guttata) (12.31 %).

Percentage of other fish was 3.81% from the total 13897. These were mainly Common carp (Cyprinus carpio) (81.13 %), Rutilus rutilus (Roach) (9.43%), Clarias gariepinus (6.60%), Abramis brama (Common bream) (2.83%).

Percentage of other basic research was 0.90 % from the total 41792. These were mainly surgical adhesion (28.80%), sport science (24.53%), investigation on life cycle of parasite (17.33%), Trichinella investigation (14.93%), protein utilization (8.00%), gene conservation (5.33%), urinary system (1.07%).

The percentage of other product types were 4.55% from the total 88 routine production uses. Were all linked to the flu.
The percentage of other efficacy and tolerance testing was 0.06% from the total 70298 regulatory uses. These were general tolerance and immunogenicity (68.29%), local tolerance (21.95%), testing of a medical laser device for dermatology use in minipigs (9.76%).

The percentage of other quality controls was 1.86% from the total 35832. This was checking the specificity of antibodies (668).

The percentage of other toxicity/safety testing was 2.66% from the total 34425 toxicity. These were mainly safety testing (65.83%), determination of the causes of fish mortality in ponds (12.12%) and investigation of presence of botulotoxin in food and feed products suspected poisoning and infection involving pet food, pet food flavour enhancer, human serum etc. (22.06%).

(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:

We would like to correct certain data supplied by Hungary in 2020. The correction regards the table 'Routine production uses by product type' and within that, the row 'Monoclonal antibody by mouse ascites method' where a value of 66 is given. This is an erroneous number as in reality there was no use of the ascites method in 2020 in Hungary. The error was due to the use of an older version of the reporting excel sheet by the firm that provided this figure. In the older version a single category was available for recording monoclonal antibody production:'[PR52] (Regulatory use/ Routine production) Monoclonal antibodies'. The data provider chose this category as the sole option, but they did not use the ascites method. In the more recent version of the reporting excel sheet however, a new category appeared, '[PR54] (Regulatory use/ Routine production) Monoclonal and polyclonal antibodies (excluding ascites method)' and the former [PR52] code was transformed to 'Regulatory use/ Routine production) Monoclonal antibodies by the ascites method only'. As the national data were transferred to the Commission report (which relied on the newer sheet) by their codes the figure of 66 appeared under the latter heading. We apologize for this fault.

Some examples:

Intravenous pentobarbital-Na injection, if necessary after premedication with XYLAZINE-KETAMINE combination Intravenous or intracardiac pentobarbital-Na injection. A total of 30 in vivo skin irritation/corrosion studies were performed.

In 24 of these cases, an in vitro study (according to OECD 439, OECD 431 and/or OECD 438) was performed at our site prior to the in vivo study to screen for irritant/corrosive properties. In 2 cases, no in vitro study was performed because structurally similar substances or other sarc had been tested previously.

In 3 cases, the test material was a pharmaceutical preparation or ancillary device (expected to be without irritant/corrosive effect) and therefore the test was not performed according to OECD 404 but according to ISO 10993 (where a prior in vitro test is not required and not acceptable).

1 test is a so-called "reliability" test, which is required to be performed (with a given reference compound) at given intervals by ISO 10993.
(Note: All study plans issued for an OECD 404 study include the sponsor's statement on the feasibility of the in vivo study, and all in vivo study plans are checked and countersigned by an institute's MAB member to ensure that the study fully complies with the required specifications.)

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

There was none in 2021.

Ireland

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

- There was an 11% decrease in the total number of uses of animals reported for 2021 versus 2020, with 122397 total uses of animals in 2021 compared to 137988 total uses of animals in 2020. This decrease was mirrored by an 11% decrease in the numbers of animals used for first time in 2021 versus 2020 (121,558 animals used for the first time in 2021 compared to 137,318 animals in 2020).

- There was a 23% increase in the number of animals reused in 2021 versus 2020 (825 animals reused in 2021 versus 670 animals reused in 2020). Cattle are the species most commonly reused in Ireland, and there was an increase in the number of re-uses of cattle in agricultural studies in 2021. The number of reuses of cats and dogs reported in 2021 also increased; this is primarily a result of increased volumes of research and development and regulatory testing of veterinary medicinal products for companion animals and an improved understanding of reuse versus continued use, leading to more accurate reporting of reuse.

- Mice remain the most commonly used species at 75% of all uses. There was a 19% decrease in the number of mice used, with 91,497 mice used in 2021 versus 113,209 in 2020. The main driver of this reduction is the 20% reduction in the number of mice used for Regulatory testing (including batch safety and potency testing) noted in 2021.

- There was a 38% increase in the numbers of rats used, with 17,050 rats used in 2021 versus 12,330 in 2020. This was due to an increase in the number of rats used for Regulatory testing (batch potency) testing of a human medicinal (biological) product.

- There was a significant increase in the number of guinea pigs used, with 774 used in 2021 versus 228 in 2020. This was a result of increased levels of Regulatory (batch potency) testing of vaccines during 2021.

- Ongoing efforts to implement non-animal methods for the safety testing of medicinal products have contributed to a significant decrease (37%) in the number of uses of rabbits reported for 2021 (657) compared with 2020 (1043).

- There was a 3.5 fold increase in the number of dogs used in 2021 versus in 2020 (from 24 in 2020 to 83 in 2021). These changes are a result of increased volumes of research and development and regulatory testing of veterinary medicinal products for companion animals.

- Reported uses of ferrets almost doubled from 186 in 2020 to 358 in 2021 as a result of increased levels of regulatory testing of influenza vaccines in 2021.

- There was a 92% reduction in the number of uses of horses reported in 2021 due to the conclusion of a large equine disease prevalence study in 2020.

- The number of uses of pigs reported in 2021 increased by almost sixfold from 2020 as a result of a large-scale study on porcine nutrition and anti-microbial resistance commencing in 2021.

- Uses of sheep in 2021 increased more than twofold versus 2020. This is as a result of increased volumes of research into the reducing the environmental footprint of the agricultural industry being undertaken in Ireland during 2021.

- No uses of zebrafish for research and testing purposes were reported in 2021. All 607 zebrafish used in 2021 were used for the creation and maintenance of genetically altered lines to generate zebrafish embryos for the purpose of 'Translational and applied research – human sensory organ disorders (skin, eyes and ears)'. The zebrafish reported are considered a harmful line, and therefore this contributed to an increase in the number of animals used for creation and maintenance of genetically altered animals with a genetic status of 'genetically altered with a harmful phenotype'.

- The number of uses of fish under the species category 'salmon, trout, chars, and graylings', increased from 0 in 2020 to 4986 in 2021. This is because this species category was not available for pre-2021 data, and so salmon and trout studied in conservation/species management/aquaculture studies would have previously been reported under the species category 'other fish'. This also explains why the number of animals reported under the category 'other fish' shown a significant reduction from 2020 to 2021.

- There was an increase in the proportion of animals other than non-human primates reported as 'born in the EU but not at a registered breeder'. This increase relates to the use of non-Annex I species such as salmon, cattle and sheep in research studies during 2021. There was also a very significant (fortyfold) increase in the proportion of animals other than non-human primates reported as 'born in the rest of Europe'. This is as a result of Brexit and the UK leaving the EU, as a high percentage of animals that are imported into Ireland for scientific purposes are sourced from the UK.

(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' increased by 29% from 2020. This is most likely due to research projects that had been paused or delayed due to the pandemic recommencing in 2021 as public health restrictions were lifted.

- There was a 20% decrease in the number of animals used for 'Regulatory Use and Routine production' in 2021 (82,325 animals) versus 2020 (102,861 animals). This reduction in the overall number of animals used for regulatory testing was driven by a significant reduction in the number of animals used for the secondary purpose 'Quality Control – batch potency testing'. 29,588 fewer animals were used for batch potency testing in 2021 (70,896 animals) than in 2020 (100,484), representing a 30% decrease. There was a notable (eight-fold) increase in the number of animals used for the secondary purpose 'Quality Control – batch safety testing' from 2020 (1304 animals) to 2021 (10674 animals). This is due to the roll-out of COVID vaccines to the marketplace, and the requirement for safety testing using animals as a condition of authorisation for these vaccines. There was a 30% reduction in the number of animals used for pyrogenicity

testing (657 in 2021 versus 943 in 2020). This reduction is the result of ongoing efforts to replace the use of the pyrogen test with non-animal alternatives.

- There was a 118% increase 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 increase was driven by two factors;

  (i) increased volumes of agricultural research investigating approaches to reduce greenhouse gas emissions and nitrate excretion from farmed animals, in order to reduce the environmental footprint of the agricultural industry, and

  (ii) The conduct of two large-scale studies aimed at protection of the marine environment and conserving and managing the Irish salmonid population.

(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 2021 is broadly similar to that of 2020, 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 numbers of uses of animals for these types of tests in 2021. For example, we have noted a reduction of 20% in the numbers of animals used for 'Regulatory Use and Routine production' overall in 2021 versus 2020, driven by a significant reduction (30% decrease) in the number of animals used for the secondary purpose 'Quality Control – batch potency testing'. 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 58% decrease in the numbers of animals used overall for regulatory testing (82,325 animals used in 2021 versus 194,816 animals in 2017), with a 63% decrease in the numbers of animals used specifically for 'Quality Control – batch potency testing' in 2021 70,896 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 severity animals experience, which is particularly important in relation to reducing the numbers of animals experiencing severe severity.

- As a result of ongoing efforts to replace the use of the pyrogen test as a product safety test with non-animal alternative tests we have noted a 30% reduction in the number of animals used for pyrogenicity testing.

- Furthermore, throughout 2021 we shared regular communications (quarterly newsletter as well as direct correspondence with relevant stakeholders) on 3Rs topics and developments with end-

users, including on the EURL ECVAM reviews of advanced non-animal models in biomedical research.

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

With regards to species, 'other' species accounted for <1% of animal use. These relate to wildlife/marine-life and environmental conservation studies using e.g. wild fish species (e.g. shark and tuna species), squirrels, and wild 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:

657 rabbits were used for pyrogenicity testing in 2021. Alternative testing methods to determine the pyrogenicity of medicinal products are recognised under the legislation of the Union. However, these methods are not suitable for certain classes of medicinal products, for example some blood/protein-based medicines. 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.

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 2021.

Italy

The data for Italy for 2021 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 an increase in fish use in numerical and percentage terms (excluding the species zebrafish, which remains constant)

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 uses for other fish species.
Note, in particular, the use of sea bass for translational research for the study of diseases affecting this animal species.

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

The trend of an increase in the 'severe' suffering level is confirmed.
Users' deployment 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 particular efforts to promote the principle of replacement, reduction and refinement and its impacts on statistics if any:

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.

The above activities contribute, in part, to the decrease in the number of animals used for scientific purposes.

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

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

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

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 440/2008 and 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).

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.

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:

In 2019 competent authority has approved 7 projects, in 2020 – 12, but in 2021 - 9. The total number of animals used for scientific purposes decreased in recent years - 4145 in 2019, 4002 in 2020 and 3741 –in 2021. During the 2019 and 2020 active licence had 29 and 34 projects respectively, but in 2020 –40 projects. However, it does not mean that in all projects all procedures were performed, and all projects were realised as planned. In some cases, projects or procedures were stopped for a while because the lack of financing or additional research before preclinical trials. Year by year the science quickly develops and that is why researchers after getting new information concerning their research topic uses *in vitro* methodology as much as possible, and it results with decreasing total amount of animals. In most of cases, especially in long lasting projects (5-year projects), researchers use less animals as they have written down in project licence application.

In 2021 significantly increased the total use of genetically altered animals with (n=491) comparing to previous year (n=281), and increased uses of animal with a harmful genotype too (n=104 in 2021, n=10 in 2020). The reason for this trend is more active research for genetically altered model animals that could be suitable for using in new drug efficiency research.

In 2018. 315 genetically altered animals were used per year, however in 2019 -109 (total decrease from 7.1% in 2018 to 2.6 % in 2019), but in 2020 the number of genetically altered animals increased to 281 (7.3%) as well as in 2021– 491 (13.1.0%). The fluctuations of data are related to our country science peculiarities. Since our country is quite small and we have only few animal users, the total number of active project licences is small comparing to other bigger countries. As a result, the ending of one project or stoppage of the research work in some projects for a while reflects significantly in specific area of data concerning to relevant branch of research. However year by year depending the type of project genetically altered animals are used more and more. As to evaluate new substances for treatment and new methods of therapy, the pathological model is needed. As the science continuously develops, now is less harmful to use genetically altered animal as a model instead of making pathological state model using specific diet or surgery. In most of cases by using genetically altered animals we can get the very beginning of the disease/pathological state that has no clinical signs but can be detected only by specific diagnostic devices or analysis. However thus very beginning of pathological state is enough for the study and in a result animal are exposed to a less harmful procedure. Moreover, in some cases using animals as the models for studies of vaccines and immunological treatment of cancer, there is a need for model that is very close to human in some specific nuances (for example some cell receptors). Using genetically altered animals we can replace the use of species with higher sensitivity with species with lover sensitivity (for example instead of using primates is possible to use mice or rats).

This year (2021) our researchers continued to re-use rodents (n=184), that were previous used in mild procedures as animals from control groups. However, this number significantly increased compering to previous year (n=45). Using repeatedly, animals were supposed to undergo non-recovery, mild or moderate procedures, where basic research was done or new specific substances were tested and after the procedures the tissue and organs were used for further investigations or stored for using in other procedures thus avoiding unnecessary use of animals for the procurement of organs and tissues.
Mostly all animals used in procedures come from EU origin (96.4%), and the biggest part 66.5% from the registered breeder. During 2021 significantly decreased animal number used in procedures

but born not at a registered breeder – from 28.7% in 2020 to 17.0% in 2021 and appeared animals that where born in rest of Europe – 8.0% that was due to large number of wildlife studies. More detailed information concerning animals that are coming not from a registered breeder, is provided in Table 1 (see below). However, in some wildlife studies it is difficult to detect from which exact part of world migrating birds or bats are coming from especially in cases where the nesting places are not separated and do not have strict borders.

Table 1

Animals born not at a registered breeder			
Region	**Species**	**Number**	**Explanation of the need**
USA	hamsters (Syrian)	40	specific lines of animals, that is not available at the breeders in EU and Europe
European Union	mice	110	animals bred at the user establishment for their specific procedure
	wild birds	184	field survey (basic research/ethology/animal biology)
	red deer	4	field survey (basic research/ethology/animal biology)
	bats	266	field survey - basic research/ethology/animal biology, Trans./Appl. Research- Animal Diseases and Disorders
	cats	299	epidemiological and basic research studies
	pigs	7	nonrecovery procedure (higher education or training for the acquisition, maintenance, or improvement of vocational skills)

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

Comparing last five years the total amount of used mice have decreased from 5289 in 2017 to 1967 in 2021 however total amount of rats varies in each year depending from type of project, but in general the total amount of used rats decreases (see Fig.1). In some specific investigations or testing of new substances rats were preferred because of their size. Rat's bigger size comparing to mice allow researchers to get more biological samples (for example – tissue, blood samples or tumour cells) for *in vitro* testing and in the same time also allow to use less animals and get more necessary data.

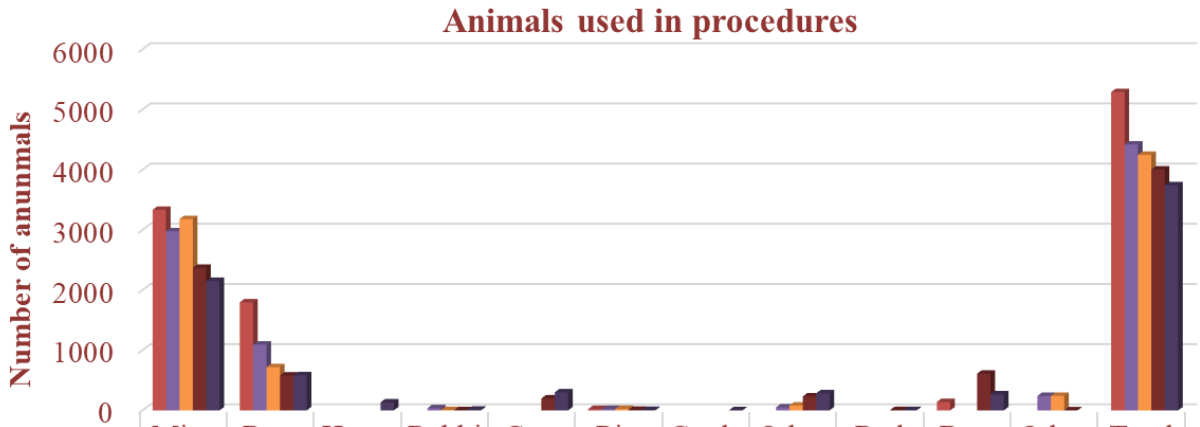
In this year continued to decrease number of used pigs – respectively from 24 in 2019 to 7 in 2021. As these animals are used only in one procedure (for higher education or training for the acquisition, maintenance, or improvement of vocational skills), the decreasing is mainly related to peculiarities of the project process.

Also, this year significantly increased using of other birds which can be explained by much more actively implemented procedures in projects that already have licences from previous years. However, the total planned number of animals in each project and procedure was not exceed. This

year also decreased the total number of used other mammals (in this case – bats) that is explained by ending of several previous projects. As we see from Fig. 1, the use of mice is falling each year. The main reason for dropping the total number of used mice is related to the development of science and more and more careful planning of procedures, gradually including more and more repeated use of animals.

In 2021 significantly increased the total number cats, which were all used in mild procedures mostly in epidemiological studies (coronaviruses and covid-19) as well as eye physiological studies. The main harm for these animals was taking a sample of blood, nasal discharge, and tears. After sample taking all these animals were immediately returned to their owners. In most of cases owners participated in procedures and procedures were performed at home to minimize stress.

Figure 1



	Mice	Rats	Hamsters	Rabbits	Cats	Pigs	Cattle	Other birds	Red deer	Bats	Other Fish	Total
2017	3332	1795				22				140		5289
2018	2975	1093		36		24		49			240	4417
2019	3178	717		6		24		80			240	4245
2020	2368	578		2	198	10		232	4	610	0	4002
2021	2151	584	132	12	299	7	2	284	4	266		3741

In 2021 researches started to use a new species – Syrian hamsters (n=132) and cattle (n=2). Syrian hamsters were used in studies related to Covid-19 (pathophysiological process and development of new drug substances). Two cattle were used in studies of new intraruminal device that monitors the physiological processes of the rumen and thus can serve as a preventive measure to prevent rumen diseases in time.

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

The biggest part of all animals was used in **mild procedures** (see Fig. 2 and Fig. 3) – 65.0.% (n=2432) from which 2049 were used in planned mild procedures, but other animals that were supposed to undergo moderate or severe procedures for the following reasons underwent mild procedure.

1. For 141 mice (purpose – Trans/Appl Research) Human Cancer)) the severity of the procedure was mild instead of moderate because no additional discomfort, stress or other changes were observed from tumour growth to animals during the experiment.

2. 78 mice (purpose – Basic Research) Cardiovascular Blood and Lymphatic System)) were supposed to be used in moderate procedure due to breeding genetically altered animals for further research in this procedure. As the phenotype in later stages of life turned out not to be harmful, animals did not suffer as researchers predicted before.

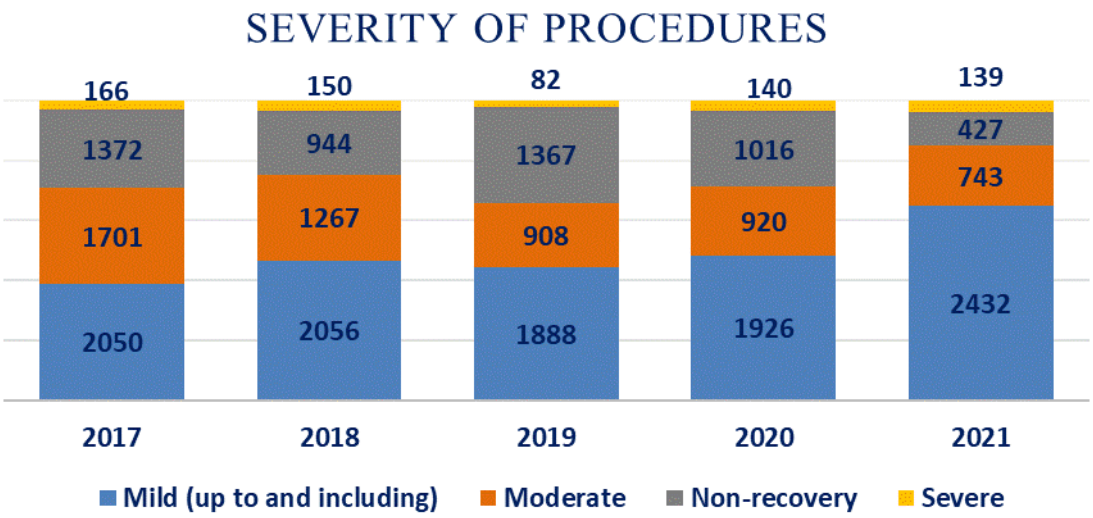
3. 10 rats (purpose - Trans/Appl Research) Human Cardiovascular Disorders)) were supposed to be used in severe procedure, but as a control group they did not receive monocrotaline therapy.

4. 45 mice (purpose -Basic Research) Musculoskeletal System)) and (purpose -Basic Research) Cardiovascular Blood and Lymphatic System)) were supposed to be used in moderate procedure due to breeding genetically altered animals for further research in this procedure. As these animals were not genetically altered and were used only for breeding purposes, they were not exposed to any additional sufferings or discomfort.

5. 8 rats (purpose -Basic Research) Oncology)) were supposed to be used in moderate procedure, but as animals from control group were not exposed to harmful manipulations.

6. 100 mice (purpose -Trans/Appl Research) Non-regulatory toxicology and ecotoxicology)) were supposed to be used in severe procedure, but at the tested doses, the compounds did not cause any toxicity.

Figure 2



In total 139 (3.7%) animals in 2021 were used in **severe procedures** from which 55 mice and 84 rats were used in planned severe procedures, but other animals unexpectedly felt severe sufferings.

1. During planned moderate procedure (purpose - Trans/Appl Research) Human Infectious Disorders)) 4 mice developed anaphylactic shock 30 minutes after the second subcutaneous administration of a new vaccine candidate.

2. During planned moderate procedure (purpose - Basic Research) Musculoskeletal System)) in 4 mice due to breeding process of genetically altered animals for further research in this procedure, early neonatal lethality of homozygous animals was observed (previously unknown).

3. During planned moderate procedure (purpose – Trans/Appl Research) Human Infectious Disorders) one Syrian hamster died from the induced infectious disease.

4. During planned moderate procedure (purpose – Trans/Appl Research) Human Cancer)) during experiment one mice lost weight more than 25 % and therefore animal was humanely killed and was not included in further manipulation

In **moderate procedures** in 2021 were used 743 (19.9%) animals from which 500 mice, 98 rats, 131 Syrian hamsters, 12 rabbits and 2 cattle were exposed to previous planned moderate procedures.

In 2021 in **non-recovery procedures** were used 427 (11.4%) animals from which all animals were used for planned non-recovery procedures with following purposes:

a) Higher education or training for the acquisition, maintenance or improvement of vocational skills – 7 pigs,

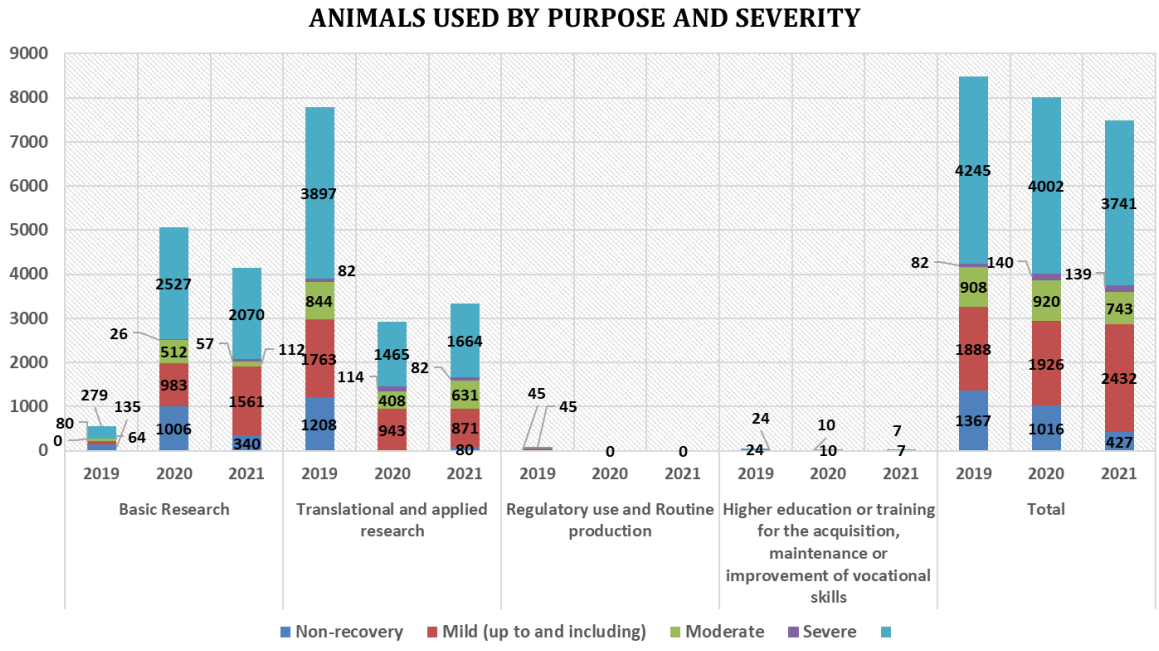
b) Basic Research (Nervous System) – 47 mice and 65 rats,

c) Basic Research (Cardiovascular Blood and Lymphatic System) – 10 mice and 90 rats,

d) Trans/Appl Research (Human Nervous and Mental Disorders) – 30 mice

e) Trans/Appl Research (Human Gastrointestinal Disorders including Liver) – 50 rats.

Figure 3



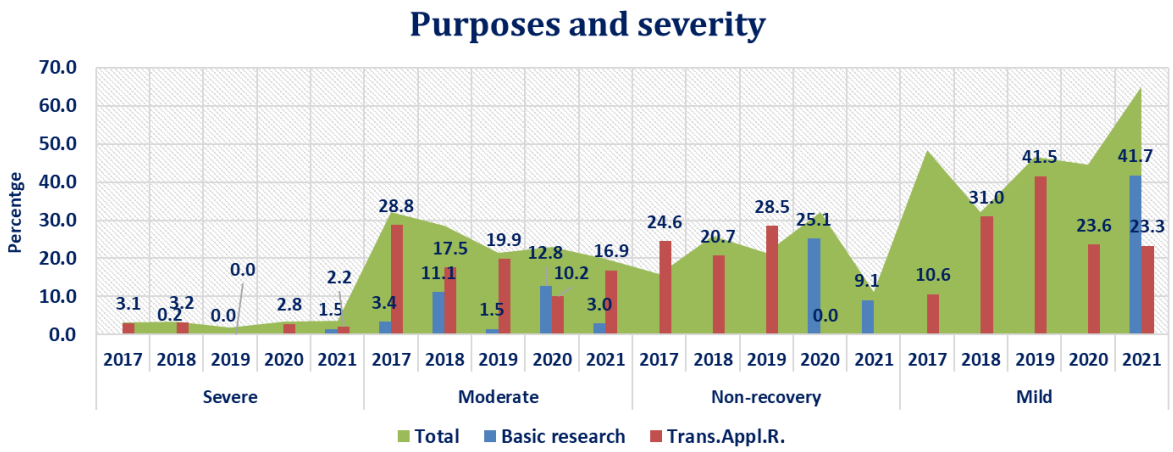
In general observation there are evident changes in severity of procedures. Comparing previous year in 2021 increased animal using in severe procedures (from 1.9% in 2019 to 3.7% in 2021), but decreased in moderate procedures (from 21.4% in 2019 to 19.9% in 2021) and in non-recovery

procedures (from 32.2% in 2019 to 11.4% in 2021). Significantly increased animal using in mild procedures (from 44.5% in 2019 to 65.0% in 2021. This is explained by more careful planning work of procedures and using of more genetically altered animals instead of model animals for several illnesses. As the technologies develop more and more, there is no necessary to use animals in late stages of illnesses. In some cases there is a possibility to detect illnesses in stages when even clinical signs are not observed, but laboratory indicators are evident. In these cases, laboratory indicators can be used testing new substances with probably preventive and/or therapeutic purposes

The main research purpose is basic and translation and applied research. This is explained with trend between researchers mainly to devote their activities to investigation of new substances or vaccines with therapeutic effect as well as to better understand some infection and performing of some pathological state (stroke, trauma, cancer, metabolic disorders), including its genetical level.

During the last year in these research branches (basic and translation and applied research) were seen changes (see Fig. 3 and Fig.4). Comparing last year with 2020, proportionally decreased animal using in and translation and applied research (from 55.3% in 2020 to 44.5%$ in 2021) but increased in basic research (from 55.3% in 2020 to 62.8% in 2021). However, last year in translation and applied research field decreased total amount of used animals. The biggest part of animals was used in basic research purposes such as Developmental biology (n=550), Cardiovascular Blood and Lymphatic system (n=396), Immune system (n=273) and Musculoskeletal system (n=200). In translation and applied research the main purposes for animal using was Human Infectious Disorders (n=606), Human Cancer (n=391) and Human Nervous and Mental Disorders (n=266). Comparing to previous year's animal using in directions of science mentioned above decreased as well as decreased a total animal using for Basic and Translation and Applied Research. These changes of animal using in specific scientific directions were related to ending of previous year projects and starting new ones.

Figure 4



Reason for animal amount changes mentioned previous (tendency to decrease animal using procedures from 2020 to 2021) is a result of scientist more carefully planned work and choosing new alternative methods and/or more thorough work before *in vivo* research that results with less and less animal need and several previously used steps – safety and efficiency tests are done without presence of animals. During the continuous scientific work researchers are looking for new alternative methods and ways to minimize animal using in procedures as well as project evaluation commission suggestions concerning 3RS principles are taken in notice. Moreover, project authors strive to use more *in vitro*, *in silico* and *ex vivo methods* (for example – isolated organs, cells or organelles instead

of live animal using), especially for toxicity and effectivity first stage tests. As well as scientists uses organs and tissue from animals that were used in other procedures as a control group animal after euthanasia.

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

Authors of scientific projects strive to use *in silico*, *in vitro* and *ex vivo* methods in substance testing processes to detect most effective sample before animal using as well as explore literature and collaborate with other scientists doing research and use other surveys to avoid repeated studies and to use as little as possible animals in procedures. During the project evaluation process competent authority and experts ensures and verifies the project scientific utility and benefits, analyse possibility to replace animals with alternative methods as well as evaluate presented animal amount in procedures and research methods and techniques. Competent authority and experts verify whether it is possible to achieve the objectives pursued in project according to the project plan. If there are any possibility to decrease animal sufferings or to decrease a total amount of animals in procedures, applicants are strictly obligated to make changes in project before authorization. In addition – during inspections each project is checked according to approved methodology.

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

Table 2

Other species used in research			
Purpose	**Species**		**Number**
	Latin	**English**	
Trans/Appl Research Animal Diseases and Disorders	*Pipistrellus nathusii*	Nathusius' pipistrelle bat	266
Basic Research) Ethology / Animal Behavior /Animal Biology	*Cervus elaphus*	Red deer	4
Basic Research) Developmental Biology	*Pipistrellus nathusii, Eptesicus nilssonii*	Nathusius' pipistrelle bat, Northern bat	410
	Sylvia atricapilla	Eurasian blackcap	154
	Sturnus vulgaris	European starling	130

In 2021, as the project was continued from 2020, 17 pigs were used in non-recovery procedures for higher education purposes (human and veterinary surgeons training). After procedure (surgical intervention) pigs were euthanized. As much as possible manipulations (cuts, trainings of surgical techniques) were done with each animal under anaesthesia and narcosis to decrease a total amount of animals.

Also, in 2021 several other mammals and birds were used for Basic and Trans/Appl Research purposes (see Tab.2)

(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 precedents for the use of animals in such categories.

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

- **Species:**
- **Numbers of animals: 0**
- **Whether exceeding the 'severe' classification was pre-authorised or not:** During the year 2021 there have not been any cases or detected information from users that the 'severe' classification was exceeded in any of procedures.
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:** During the year 2021 no procedures exceeding 'severe' were carried out.

Lithuania

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

In 2021, there were 5802 laboratory animals used for scientific or educational purposes in Lithuania. In comparison to the previous year 2014 (were 3788) more animals were used in the projects.

The number of users increased from 8 in 2013 to 12 in 2015 to 14 in 2017 to 15 in 2020 to 16 in 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 most common primary purpose for using animals was "Basic Research" (~ 8,5 % increase, 2740 animals was 2524), then Trans/Appl Research (~ 22 % increase, 1185 animals was 972), for the purpose Higher education 84,5 % increase / 1877 animals was 292).

The increase of the animals was used in the Higher education is the use of the fish (Oncorhynchus mykiss, Salmo trutta fario, Salmo trutta trutta, Gasterosteus aculeatus, Sander lucioperca, Esox Lucius, Serinus canaria).

Some approved establishments did not perform any projects in 2021 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 (~49,7 %) were used for the procedures classified as mild [up to and including] severity, non-recovery (~ 36,7 %), moderate (~ 13,6 %), severe ~ 0,03 %.

Animals for the procedures classified as mild [up to and including during year 2021 was used 2881, non-recovery 2128, moderate 791 and severe 2 animals.

The 'severe' classification reported in 2021 consists of 2 animals (Mice (Mus musculus) 0,03 % of the total number of used animals. In the project "The evaluation of the treatment efficiency of electromagnetoporation combined with chemicals and dendritic cell vaccines for the treatment of mouse tumors ", the tumor caused during the procedure caused pain to the animals, prevented them from moving. The animals were killed.

(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 310), Salmo trutta fario 154, Salmo trutta trutta 130, Gasterosteus aculeatus 412, Sander lucioperca 300, Esox Lucius 480, Serinus canaria 147 were used during the reporting in 2021.

(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 has been performed not involving the use of live animals in accordance with Directive 2010/63/EU

(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:**

The 'severe' classification reported in 2021 consists of 2 animals (Mice (Mus musculus) 0,03 % of the total number of used animals. In the project "The evaluation of the treatment efficiency of electromagnetoporation combined with chemicals and dendritic cell vaccines for the treatment of mouse tumors ", the tumor caused during the procedure caused pain to the animals, prevented them from moving. The animals were killed.

No exemptions under article 6(4)(a) of Directive 2010/63/EU were granted in 2021.

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, 5341 animals in 2020 to 4967 animals in 2021. This decrease may be a consequence of the reduction of the research activity during the lockdown and the sanitary measures caused by the Covid-19 pandemic.

Concerning the distribution in the species, the main reduction results by the decrease in use of mice in procedures, notably in 2021 a total of 4114 mice were used compared to 4775 mice used in 2020. There is a increase of the use of zebrafish from 825 zebrafish in 2021 compared to 556 zebrafish in 2020.

Between 2020 and 2021, the use of rats in procedures was rather stable.

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 decrease in the total number of uses and in the total number of uses in mice could be observed between 2020 and 2021.

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 from 2020 to 2021, 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 encouraged the users and the members of the animal welfare bodies to participate in 3R webinar focusing on the replacement, reduction and refinement during the lockdown caused by the Covid-19 pandemic.

- 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 2021, there was no cases 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 2021 a total of 423.650 uses has been registered, which is a rise of 4% with regards to 2020 (406,799). A rise of this size fits the general fluctuations observed over the last 5 years.

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

Significant increase have been found for: other fish (new field research projects started), Rabbits (increased demand product), hamsters (Covid-19 research), Salmon/trout/chars/graylings & Guppy/swordtail/molly/platy (new categories), other rodents (new projects registered), dogs (project into the spread of Covid-19 amongst dogs), cephalopods (new research with species), seabass & turkey (new categories), Resus monkey (Covid-19 research).

Significant decrease has been found for: other birds (decreased project capacity), guinea pigs (decrease in basic research), cats (end project into spread Covid-19 pet cats), reptiles (end of project).

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

In 2021 the Netherlands has seen a change in case severities of which a rise in moderate severity from just shy of 31% of the total up to 36,6% of the total is the most noteworthy, most likely linked to research into Covid-19. This change reflects a total of 27.110 uses more in the moderate category than the previous year.

(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:

The Netherlands registers a significant amount of 'other fish', which is mostly Anguila anguila and Fathead minnows. The Netherlands also registers a significant amount of 'other birds', which is mostly Parus major, Ficedula hypoleuca and Turdus merula. The use of both categories can largely be attributed to field research.

Our efforts to reduce the unnecessary use of the category 'other' in research purposes has seen it's result in the 2021 registration. The changes in main categories can partially be attributed to increased efforts to use the categories provided by the ALURES database.

(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 37 records, with a total of 581 uses, have been registered. As explanation for the use of animal testing the majority of entries is a result of *in vivo* test being required after a positive result from an *in vitro* assay or for model validation according ICH S10 guidelines.

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

In 2021 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:

In 2021, the slight overall downward trend in the number of animals used in Poland continued. However, following the COVID-19 pandemic which saw a lot of testing suspended in 2020, testing has been slowly increasing to previous levels in some experiment groups.

(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, for example, orders from external parties. For example, the use of horses to research animal diseases and disorders increased significantly in 2021.

(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 during the approval of experiments and not only the severity described by the competent authorities (local ethics committees on animal experiments), there appears to be a downward trend in the number of animals being used in severe and moderate procedures, with an increase in mild procedures. This trend confirms the possible overestimation of the severity of procedures in experiment approvals. Animal welfare groups 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. Their activities are monitored by the KKE, which prepares a comprehensive analysis of their activity reports.

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

In 2021, 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. All experiments on amphibians, over 50% of experiments on birds, and almost 20% of experiments on fish are conducted on wild animal species.

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). This user tests herbal medicinal products and in 2021 its activities in Poland accounted for over 70% 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 2021, such cases were identified in 11 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. Testing was carried out in accordance with standard
    a. PN-EN ISO 10993-11:2009 – Tests for systemic toxicity (5. Acute systemic toxicity. Pharmacopoeial testing). An alternative method exists which does not involve animals. However, as Annex G to ISO 109993:11 points out 'The LAL test is not suitable for determining the pyrogenicity of these substances'.
    b. PN-EN ISO 10993-10:2015-02 – Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization, which states that there are no satisfactory in vitro alternatives to replace in vivo testing. Irritation testing of medical devices in accordance with the applicable standard is required by notified bodies.
    c. PN-EN ISO 10993-11:2017, which allows the use of rodent species (mice or rats) for acute oral, intravenous, dermal and inhalation studies of medical devices.
    d. PN-EN ISO 10993-23:2021-08, which is a non-clinical standard test of medical devices required for registration purposes before placing them on the market.

The aforementioned ISO 10993 European standards have been approved by the European Committee for Standardization for irritation and sensitisation testing of medical devices.

3. Since some substances may also contain non-endotoxin pyrogens that are not detected by the BET method, a test covering all types of pyrogens is required to confirm the absence of non-endotoxin pyrogens (European Pharmacopoeia General Chapter 2.6.30). The monocyte-activation test (MAT) has been added to the European Pharmacopoeia, providing an in-vitro alternative to the RPT that is

capable of detecting both endotoxin and non-endotoxin pyrogens. Once this method has been validated in terms of the antitoxins produced and following its approval by the Office for Registration of Medicinal Products, rabbit pyrogen testing will no longer be performed. Until the method is approved by the Office for Registration of Medicinal Products, rabbit pyrogen testing to detect the presence of antitoxins (viper venom antitoxin and botulinum antitoxin) is a legal requirement.

(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 2021, no such cases were found.

Portugal

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

In 2021, there was an increase in animal use compared to 2020. The total number of animals used in 2021 is 82406, which constitutes a 19,95% increase in animal use, compared to 2020 (total uses: 65966).

This figure includes the number of animals used for the creation (2025 animals) and the maintenance (2546 animals) of genetically altered lines which represent 5,54% of all animal uses.

In the uses of animals for research, testing, routine production and education and training purposes (total uses: 77835), mice continue to be the most used animal species (71,47%), followed by Xenopus (8,70%), Sea bass (7,39%) and Rats (7,02%).

There is a decrease in the use of Fish (total uses: 9292) and an increase of use of Mammals (total uses: 61633) and of Xenopus (total uses: 6777).

No cephalopod was used in 2021.

There was an increase of 1,31% on reuse of animals (total reuses: 2783) compared to the previous year (total reuses: 1431).

Basic research (61,23%) continues to be the most frequent purpose for which animals were used to, followed by translational and applied research (37,35%), and by Training for the acquisition, maintenance or improvement of vocational skills and Higher education.

The use of animals for Regulatory and Routine production only represents 0,11% 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:

The decrease of animal use that occurred in 2020 due to the COVID pandemics, was reverted in 2021, which was noted with the general increasing in the performance of procedures, those that had to stop being performed for that reason.

Xenopus is, indeed, the animal species that showed a higher increase, of 8,71%, compared to the previous year, which was due to the performance of procedures related to studies of developmental biology and of non-regulatory toxicology and ecotoxicology.

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

No change in the trends of actual severities was noted, i.e., in 2021 (and since 2017), the trends in the proportions of animal use by actual severity experienced is reported in the following categories, from the highest to the lowest use:

- Mild: 39, 10%
- Moderate: 37,19%
- Severe: 18,35%
- Non-recovery: 5,26%.

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 decrease in the use of Other fish in 2021 (total uses: 1535), compared to 2020 (total uses: 8920) but the main justification for that is the update of reporting data in the new excel file, which was modified in order to include new specific entries for specific animal species that were previously considered as belonging to the Other fish entry.

In the entry of Other fish, and among the several species that were reported of having been used, *Sparus aurata* and *Anguilla Anguilla* were the most used animals species, representing, respectively, 63,52% and 27,36% of all uses of this entry.

There was a reported increased in the use of Other mammals (total uses: 73), compared to the previous year, belonging to studies of Animal biology with the following animal species:

- *Globicephala macrorhynchus*
- *Physeter macrocephalus*
- *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 major increase in the number of animals used for scientific purposes for the first time, from 7586 in 2020 to 14858 in 2021 but this is just because 2020 was unusual, due to the COVID-19 pandemic, with a low number of animals used. Therefore, there has been a slight increase compared to the years before 2020 (11680 animals in 2018, 13292 animals in 2019).

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

Overall, when compared to 2020 during which there has been a small number of projects as a result of the pandemic, a large number of studies were performed in 2021. In particular, there has been an increase in the proportion of animals used for basic research purposes, mostly explained by a rise in the category of Nervous system and Endocrine system/Metabolism. Although the number of animals used for translational and applied research was higher than in 2020, its percentage out of all uses dropped. A significant number of animals were used for quality controls for vaccines and other biological medicines. However, no batch potency studies were carried out.

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

There has been a very significant drop in the non-recovery uses mainly due to a better assessment of the degree of severity of the procedures (e.g. for diagnostic tests for diseases). Following a training organised at national level last year, it became clear that the severity should not be categorised as "non-recovery" if animals regain consciousness, even if humanely killed at the end of the procedure.

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:

No animals have been used in high education as they were replaced by other techniques in teaching. Apart from the annual meeting of our National Committee, a training was organised at national level promoting the principle of 3R. Where procedures allowed, prior in vitro tests were used.

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

A significant number of animals has been reported under "other" category mainly due to studies for the quality control of the immunity value and innocuity value of medicinal products for veterinary 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:

25 rabbits were used for testing the pyrogenicity of the pharmaceutical substances (medicinal products for human use) as the beneficiaries are in countries that accept only the Pharmacopoeia of the Eurasian Economic 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:**

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 the previous reporting year, the overall usage of animals in research decreased by more than 30%. This decline is primarily due to the limits imposed by the COVID 19 pandemic, which forced several research groups to cease research studies and postpone projects. Additionally, during the COVID-19 pandemic crisis, grant submissions for animal studies were limited. With a few exceptions, many animal users, breeders, and suppliers in this region were unable to attend the job and were forced to work remotely.

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

We observed a nearly 50% decline in the use of animals in neuroscience research in 2021. On the other hand, compared to year 2020 we saw an almost 67% increase in the use of animals in immunology. The COVID-19 pandemic may have had an impact on this pattern, given the research focus on the pathogenesis and research towards effective therapy of this disease. One of the most prominent areas affected was the discipline of "Ethology / Animal Behaviour /Animal Biology", with 93% drop in the number of animals used during year 2021 in comparison to the previous year. This significant change was caused by the completion of the projects in the respective research area this year.

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

We noticed a nearly 38% increase in severe severity, which can be linked to increased regulatory testing and routine manufacturing. In particular, over half of this increase is due to reporting combined endpoints for the first time, which raises the level of severity for the animals utilised in the study. Specifically, it combines two types of studies: repeated sub-chronic 28-day injection of the reproductive component and evidence of endocrine disruption. It combines OECD 407, OECD 443, and OECD 414, 415, 416. Even though such study cannot completely replace all of these assessments, the data gained can be used to determine the reproductive/developmental implications of recurrent therapy while using fewer animals.

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

Annually, our experts along with others who work with animals get together to review animal practices as well as any new trends or advances in the field. Alternative techniques are also promoted, primarily through the efforts of the Slovak national platform for 3rs. Furthermore, at every given stage, applications for ethical approval are evaluated using the 3R principles. Applicants who do not adhere to this 3R principle ought to comply with the guidelines. Furthermore, the three Rs, the Humane Endpoint, and the pursuit of alternative techniques are consistently emphasised throughout training sessions for users, breeders, and providers, which may be one of the reasons for the consistent decrease in the number of animals used for scientific purposes in Slovakia.

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

In contrast to the previous reporting year, no substantial proportion of animals classified as "others" were used in 2021. However, we did observe more than 30% reduction in the number of birds employed in this category when compared to 2020. In contrast to the previous year, only the following species were utilised in projects in 2021: *Coturnix japonica, Lonchura oryzivora,* and *Lonchura striata domestica.* This decrease can be due to the fact, that the research group working with these species was finalizing the project.

(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:

During the project evaluation process, the applicant must adhere to the 3R principles, in this case the replacement. If an alternative method not involving the use of live animals exists, the applicant is asked to use such method. Thus animals were not used in these categories.

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

During the operational year 2021, no violations of the approved severity classifications were observed. The legislation of the Slovak Republic has developed a system of utilizing project retrospective evaluation, which is not only dependent on the project being labelled as severe, but also on the high number of animals used, the use of several complicated methods and methodologies within one project, and the testing and use of substances in the project, the findings of which from in vitro tests were not fully accessible at the time the project was approved.

Slovenia

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

Number of uses in animals slightly increased, from 5.796 in 2020 to 5.817 in 2021. More rats, pigs, sheep, and fish were used compared to previous year, although mice still represent the highest percentage (90,2%) of all used animals in Slovenia. One new species was used: 40 hamsters in basic research for immune response in development of covid vaccines.

No cats, dogs and non-human primates were used for scientific purposes in 2021.

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

Small number (55) of fish (Oncorhynchus mykiss) were used in the University educational programme for the veterinary science students. This is an ongoing programme for students where they get familiar with sedation of fish and taking blood samples. This method avoid sacrifice of fish in order to get samples for diagnostic tests. The increase in the numbers of used fish compared to previous year was probably due to covid restrictions in 2020.

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

Most procedures are classified as mild. Compared to 2020, there was a slight decrease in the number of "mild" procedures and slight increase of those classified as "moderate" and "severe". The only species used in procedures classified as "severe" in 2021 were mice and pigs. The higher number could be the result of covid restrictions in previous year and use of pigs in oncology studies. Compared to all animals used in procedures in 2021, animals used in "severe" procedures 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, regular meetings with animal welfare officers, different workshops, 3Rs awards, Innovations in 3Rs programmes, Celebration of BRAD Day, etc…

Example of reduction: mice, used for pharmacopeia test for determination of biologic activity of erythropoietin are re-used if the age of animals and frequency of samples allows.

Examples of refinement: refinement of the cages for rodents (extra nesting materials, extra wood for nibbling; refinement of feed for rabbits with hay, before only pellets were used; identification of mice with small ear tags, which are very light and don't influence the physiological position of the ear, etc.

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

55 rainbow trout (Oncorhynchus mykiss) were reported under category “other” fish which is less than 1% of all used 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:

Although alternatives exist, rabbits (68 animals) were used in pyrogenicity testing, in the field of regulatory use and routine production. The main reason is that regulatory organs still require rabbit pyrogen test when there is LER (low endotoxins recovery) effect in BET method suspected or confirmed, to show absence of pyrogenic substances. It is also required in registration process to show absence of pyrogenic substances with non-endotoxin origin, which is not possible to detect with alternative BET/MAT method. 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.

(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:

Scientific and teaching activity in 2021 has been influenced by the situation arising from the COVID-19 pandemic, which makes it difficult to assess trends in animal uses.

Throughout 2021, the number of animal uses has shot up significantly in comparison with the previous year.

The main reasons for this significant increase are twofold. Firstly, there is a significant increase in the number of authorised projects, in higher quantities than before the COVID-19 pandemic. Secondly, the development of processes at very early stages of fish development has resulted in a single project using half a million sea bass larvae which have reached the status of independently feeding larval forms. This project is the main reason for the increase in the number of uses in 2021.

In regards to the origins of the primates used, the proportion of animals originating from Africa is increasing, to the detriment of those from Asia. This is considered to be the result of the difficulty in purchasing animals from the African continent during the health crisis suffered in 2020.

In line with recent years, the replacement of invasive techniques for genetic characterisation has stayed constant, in particular distal tail-docking, as surplus tissue from identification is used.

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

Reuse has increased in 2021, probably as a result of the measures taken during the COVID-19 pandemic, which included an increase in the euthanasia of animals and therefore less availability of animals.

The new subcategories for the classification of research purposes have led to a redistribution of the classification of the uses carried out, particularly in the subcategory of applied animal nutrition research.

- **Species**
- **Mammals**
- **Rodents**

During this year, the use of all rodents has increased, with the exception of guinea pigs. The use of rats, mice and golden hamsters has particularly increased. The reason for this increase lies, on the one hand, in the recovery of pre-COVID-19 working levels, and in regards to mice and rats, also the need to recover and maintain genetically modified animal lines that had to be suspended as a result of the animal care and maintenance difficulties that occurred during the pandemic. In the case of

golden hamsters, having SARS-COV-2 receptors makes them an appropriate model for very useful research in projects on this topic. They have particularly been used in the production of vaccines.

- **Rabbits**

In 2021 they were mainly used for quality control under the regulatory production heading (as in previous years), and in the field of animal nutrition.

Genetically modified animals are being used in fertility research. Genetic modifications in rabbits will serve as a biomodel for human reproduction, as the aim is to assess the function of a gene present in rabbits and humans, but not in mice.

- **Carnivores**

In the carnivores group, compared with the previous year, there is an increase in the use of dogs for regulatory purposes, and for research into diseases and nutrition in which they are the target species.

There has also been an increase in uses in ferrets linked to studies on physiological mechanisms of the nervous system where this animal species is the model of choice.

- **Primates**

In 2021 there was an increase in reported uses compared to 2020, and the majority of them, if not half, are re-uses. In regards to the purposes, reporting was mainly under the 'regulatory' heading; this animal species is now required 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 primates.

- **Birds**

The vast majority of uses are in poultry and have increased compared to previous years partly due to the high number of animals included in the studies, where they are kept in commercial conditions.

For uses within the group 'other birds' there is a decrease in the number of uses reported, but this is not particularly related to the fact that the information on turkeys, for which the number of uses is low, has been broken down in this year's report.

- **Fish**

In 2021, the breakdown that has been made with specific entries for the salmonids and sea bass group, among others, provides a much more detailed picture of the research carried out on these species, which is of great importance in Spain, given the importance of aquaculture.

The high figures in the use of some marine species of animal production fish can at times be surprising. The explanation lies in the fact that work on these cases is mainly carried out in the study of the early stages of development of these species, their behaviour, feeding, biology and reproduction, and is carried out in the few days before they acquire the status of independently feeding larval forms and are therefore to be counted in use. In practice, they use the breeders or their

layings, starting their studies with a single laying that, for example, in the case of the sea bass may be as high as 250 000 eggs per kg of live weight.

- **Cephalopods**

The use of cephalopods is linked to the research being carried out for their rearing in aquaculture, focusing on better understanding of how to improve their wellbeing, the adequate nutrition at each stage of their development and deepening knowledge of the diseases that may affect them.

- **Purposes**

- **Basic research**

The number of uses in this field has decreased in comparison with previous years.

On the one hand, uses under the headings of oncology and nervous, cardiovascular and immune systems have increased, with a parallel increase in the use of rodents, and in particular genetically modified ones.

However, behavioural, ethological and animal biology research uses have significantly decreased. This decrease is particularly noticeable in rabbits and domestic birds due to the use of commercially kept animals in several projects for this purpose. The high number of animals that make up the flocks in these circumstances, or the groups mean that each project carried out involves a very high number of uses. Moreover, studies that have been classified as animal biology in previous years have been reported in the new animal nutrition subcategory.

Finally, the completion of projects in investigations into the urogenital and gastrointestinal system in pigs, and on the nervous system in sheep, have led to a very significant decrease in the use of artiodactyls in basic research.

- **Applied research**

Under this heading, the significant increase in the use of sea bass stands out, which makes the relative weight analysis of each applied research subcategory difficult.

This increase is largely due to a single procedure in which eggs from a single bass laying were used in a nutrition investigation. During the course of the procedure, coupled with a new feed for these animals, the fish reached the status of independently feeding larval forms.

In regards to the evolution of the research subcategories, there is generally a decrease in uses in research related to human diseases, in particular in diseases affecting the sensory organs (skin, eyes and ears), endocrine and metabolic diseases, and musculoskeletal diseases, which do not compensate for the increase in uses for infectious diseases, cancer and cardiovascular diseases.

There are also a very significant number of uses for animal nutrition purposes (over 590 000, accounting for almost 46% of all uses), a subcategory incorporated in this year. Research into fish nutrition has a vital role on account of the importance of aquaculture in Spain and the fact that there

is a great keenness to develop the technology and knowledge to enable the growth of aquaculture, so as to mitigate the decline in traditional fishing as far as possible. Furthermore, studies involving groups of animals under commercial conditions mean that the number of animals involved and their use is very high, particularly in the case of domestic birds or rabbits. This is also the case in animal welfare research (where aquaculture fish have been used mainly).

Attention should be drawn to the significant number of uses to improve animal welfare in cattle and pigs, for which the foreseeable amendments to their governing regulations may make it necessary to implement new management systems on livestock holdings.

As regards the situation relating to animal welfare research, aquaculture fish have been the most widely used species, and to a lesser extent cattle and pigs.

In 2021, cephalopods have been investigated in three Spanish centres, with the aim of expanding the knowledge available on their well-being and nutrition.

- **Routine and regulatory production**

In 2020, the Covid-19 pandemic boosted the development of vaccines and treatments for this disease and, as a result, the number of uses of animals for regulatory purposes increased very significantly. In 2021, continuing the lines of investigation already addressed in 2020, there was a marked increase in uses compared to the trend of decline which was observed up to 2020.

In regards to the species used, there is a significant decrease in the use of mice, and to a lesser extent rabbits, as well as an increase in uses of fish species intended for aquaculture, where work is being done to prevent fish diseases during farming, and where there is less experience than for other production species.

- **Routine production**

In 2021, the heading ‘other routine production’ has been segregated, introducing an obligation to report specifically on the sub-category of monoclonal and polyclonal antibody production by different methods of induction of ascites in mice.

However, for the first time, and although the number of uses in previous years was already decreasing, no mouse has been used for monoclonal antibody production by ascites in mice.

- **Regulatory production**

The upward trend in quality control, efficacy and tolerance uses continued in 2021, while there was a decrease in those aimed at ensuring product safety (toxicity and safety controls), where alternative methods are more widely available.

The main normative basis for this regulatory production is the legislation on veterinary products and their residues, in order to meet EU requirements.

- **Quality control**

Worthy of mention is the number of uses intended for batch potency testing, where the levels reached in 2020 were maintained and which represented a very noticeable increase compared with 2019.

In regards to batch safety testing, the downward trend in the number of uses that was interrupted in 2020 is restored.

There is a significant increase in the number of uses involving poultry for testing the absence of foreign agents, under other quality tests.

- **Other efficacy and tolerance testing**

The vast majority of uses have been carried out on salmonids, in efficacy tests for vaccines under research and development, to determine the degree of protection against experimental infections for salmonids.

- **Toxicity and other safety testing**

There is a continuing downward trend in the number of animal uses, especially in the use of mice.

As regards the legislation on the basis of which these tests are carried out, 40% of cases involve medicinal products for human use, 33% involve food law, including food contact materials, and only 18% are carried out in accordance with legal requirements on veterinary medicinal products and residues thereof.

The highest number of uses are carried out in food and feed safety tests, with 32% of uses in toxicity, a decrease compared to previous years (46.5% of uses in 2020) partly caused by the abandonment of the mouse bioassay for PSP toxin determination.

Secondly, repeated dose toxicity studies (22.5% of uses) are found, with a significant increase in the number of uses of crab-eating macaque resulting from a reclassification as reuse, which had previously been considered as continued use. Increases in the number of uses of dogs and pigs are linked to the development of new projects.

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

The gradual decrease in the number of uses observed since 2015 has been maintained, although, given the figures, there is a rebound in comparison with the uses made in 2020.

Again, it should be noted that in 2020 there was a very significant decrease as a result of the COVID-19 pandemic, as several projects for this purpose were interrupted or suspended.

Projects carried out for this purpose are mostly mild severity (80%), although moderate severity has been reached in two projects carried out on starlings and African clawed frogs, accounting for just over 11% of uses.

Under the heading ‘Other animal species’ used in research to preserve the environment, there have been species considered invasive, such as *Gambusia holbrooki.*

- **Preservation of species**

This heading also shows a continuous decrease in the number of uses since 2015. In 2021, uses for this purpose were made in ‘other species’, mainly birds (57% of uses), including various eagle species (such as osprey or imperial), vultures, falcons and Egyptian vultures. 34% of the uses have been carried out on reptiles.

All the uses carried out in 2021 have been of minor severity.

- **Higher education or training for the acquisition, maintenance or improvement of vocational skills**

The decrease in the number of uses has also remained constant for this purpose. The 2021 number of uses can be misleading because it may appear to be a rebound of the 2020 number of uses. However, it should not be forgotten that the major decline in animal uses in 2020 was largely due to the situation created by the COVID-19 pandemic.

The species most frequently used have been mice, rats, rabbits, and pigs.

Moreover, from 2021 onwards, the information under this heading is provided separately under the sub-category Higher Education on the one hand, and training for the acquisition, maintenance or improvement of vocational skills / skills maintenance, on the other. Thus, in 2021, 30% of uses were for higher education, mainly involving domestic birds, mice and rats; and 70 % were for training on the acquisition, maintenance or improvement of vocational skills, mostly involving rats, mice and pigs.

- **Maintenance of colonies of genetically altered animals, not used in other procedures.**

In 2021 there was an increase in the uses reported for the purpose of ‘maintaining colonies of established genetically modified animals, not used in other procedures’, possibly due to an increase in breeding activity slowed down by last year’s COVID-19 outbreak (2020) and a better understanding and registration as ‘uses’ of invasive tissue sampling techniques in genotyping of animals.

34 437 mice have been used in maintenance, of which 92% have been reported for invasive phenotyping, either they are not genetically modified or have no harmful phenotype, and only 8% have been reported due to their harmful phenotype. Of the 8%, the vast majority, 60%, have experienced mild suffering, slightly less than 40% have experienced mild [sic] suffering, and less than 1% severe suffering.

As is becoming the norm, animals were not used in **forensic investigations**.

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

In 2021, it is particularly difficult to assess the severity trends of projects, given the large number of sea bass larvae used with mild severity. As has been the case in the past, this type of project introduces uncertainty in the assessment of trends.

In any case, the downward trend in the proportion of uses without recovery remains, largely due to the continuing correction of the increasingly less numerous uses, misunderstandings as to the true meaning of the concept of 'no recovery'.

Mild-severity uses have increased dramatically, but it is considered that this large increase is the result of a one-off situation, due to a particular project focused on research into special feedingstuff for larval stages of seabass.

Both animals that experienced moderate and severe severity increased in 2021. There are several reasons for these increases. Firstly, as pointed out last year, there is greater awareness and caution in the assessment of the severity, and situations previously classified as moderate severity are now considered as severe. Secondly, the number of projects dedicated to microbiology and regulatory procedures in the manufacture of medicinal products or veterinary products has led to an increase in the severity suffered by animals. In addition, there are other projects where, despite establishing early endpoints, animals have experienced severe suffering.

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

A variety of approaches have been taken to implement the 3Rs principle, with a range of contributing actions. For example:

- Particular emphasis has been put on training staff, requiring training also for animal welfare staff of breeders, suppliers and users and responsible veterinarians. All those staff for whom specific training is required are also obliged to carry out activities to ensure that they keep their skills up to date.
- Care culture is seen as a basic pillar for promoting the implementation of the three Rs, and a specific implementation plan for this culture is sometimes requested.
- Documentary support, with the preparation of harmonised documents that facilitate a thorough follow-up of the controls to be carried out.
- Applying monitoring protocols (including corrective measures and end-point criteria) designed specifically for the procedure in question, with expected parameters according to the type of procedure and/or the animal species.
- Targeted checks are being carried out on specific aspects, allowing a better assessment of the situation and analysis of the gaps in the implementation of the three Rs.

- During the project authorisation process, more and more detail is required in the documentation associated with the procedure, in order to make it easier to verify the aspects assessed in the application of the 3Rs and, in turn, to be able to require or propose that the parties concerned make improvements in this regard before the project is authorised. In particular, it is requested to indicate the process followed to check whether or not there are alternative methods to the use of animals for the purpose of the project and the sources or keywords used for searching for alternatives, the date of the references and the search carried out.
- Training promotes the use of carcasses, organs and parts from slaughterhouses/butchers/fishmongers. Dead bodies are often used in a step prior to the use of live animals.
- Improvement or substitution of certain techniques, such as the replacement of invasive systems for genetic characterisation of animals with non-invasive systems.
- Promotion of sequential studies.
- Placing an emphasis on the correct selection of the sex of the animals in studies or their balanced inclusion.
- Stressing that end-point criteria be clearly defined for each procedure.

These measures are developed at the project evaluation and authorisation stages. Inspections at centres are also considered to be a key action to promote the principle of the 3 Rs, especially where Refinement is concerned (whereby corrective measures are to be indicated in relation to the facilities: material, dimensions, flows, biosecurity measures, environmental enrichment aspects, etc.)

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

In the assessment of the sub-sections 'other', it should be kept in mind that new sub-categories have been introduced this year to report separately on concepts previously reported on under the heading 'other'.

Nevertheless, this type of research, largely for ecological and productive purposes, among others, is often carried out in the context of animal production or species conservation and takes place outside the user centre, which sometimes makes monitoring and counting control more difficult.

The following can be reported in regards to **'other animal species'**:

The use of other animal species, which has remained above 10% in recent years, has decreased to less than 4% of the total animal uses made. In addition to the traditional variability in the number of individuals in samples of early development stages of fish and other oviparous animals, there are specific rubrics for turkeys and for several fish species.

- **Other rodents**, which accounted for 0.05% of the uses involving rodents: all uses have been made for basic research purposes, in particular in nervous system and behaviour, ethology and animal biology research. Almost half of these rodents are wood mice (*Apodemus sylvaticus).*
- **Other carnivores**, which accounted for 1.76% of the uses involving carnivores: mainly *Herpestes ichneumon* (Egyptian mongoose) have been used to protect the environment and mink in animal disease studies.
- **Other mammals.** Particularly:
    - Greater white-toothed shrew (37% of uses of other mammals), deer and beluga whales in basic research for the biology, behaviour and ethology of these animals.
    - Wild boar (16% of uses of other mammals in applied research for disease studies).
- **Other birds**, which accounted for 3.18% of the uses involving birds. As stated above, turkeys for the first time have a specific entry, although the number of uses involving this species was only 0.11% of birds.

  It should be noted that the rate of reuse, 15%, is much higher than the average of the total uses reported.
  The purposes for which they are used are mainly studies on their biology, behaviour and ethology in the field of basic research, animal disease research in the case of applied research and research for the preservation of species and the protection of the environment.
  Under this heading, uses involving wild species are reported. These are often protected species, for example Egyptian vultures, eagles (imperial, osprey), vultures, such as the griffon vulture, or blue tit or partridge.

- **Other fish**, which accounted for 6.62% of the uses involving fish in 2021. Under this heading in particular, it is difficult to assess trends because new specific headings have been introduced for sea bass, salmonids, etc.

  Most of the fish under 'Other fish' are aquaculture fish, with investigations into sea bream and sole dominating. In the case of these fish, the main aims are to study the diseases affecting these animals, their nutrition, biology and behaviour, animal welfare and vaccine development.
  Moreover, 10% of the fish under this heading are medaka, which are mainly used in multi-systemic basic research studies.

- **Other amphibians**, which have accounted for most uses involving amphibians (around 90%), carried out in centres specialising in wildlife work, with the aim of deepening knowledge of their biology, behaviour and ethology, as well as development biology.

As regards animals **'used for other purposes'** the following is noted:

In 2021, separate purposes have been introduced as separate headings from the previous heading 'other'.

- **Other basic research**

- The project started in 2019 on palatability of veterinary products (42% of uses under this heading), mainly in cats and to a lesser extent in dogs, continues.
- Pain and analgesia treatment (20%), in rats, with mild or moderate severity
- Adaptation and improvement of imaging techniques (12%), in mice

- **Other applied research**
    - Other human diseases, classified as iatrogenic, degenerative and developmental, using zebrafish and mice.
- **Other uses in routine production** (40% of routine uses): predominantly for vaccine antigen production in rabbits
- **Other efficacy and tolerance testing** Efficacy controls mainly in the development of salmonid vaccines.
- **Other quality controls** mostly reported as
    - testing the absence of foreign agents
    - control in the production of antiserum
- **Other toxicity and safety testing**
    - Diagnosis of tumour cells with safety evaluation by histopathology
    - Immunogenicity
    - Laparoscopy safety evaluation by assessing tissue heat damage
- **Other lethal methods** Maximum tolerated dose (MTD) using mice.

(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:

- **Tests for the detection of pyrogens in rabbits**

Although in 2021 (7 055 uses) there was a slight rebound in the use of animals for this purpose, compared with 2020 (7 005), these 7 055 uses represent a reduction of about 15% compared to the average uses for the period 2018-2020.
The vast majority of uses are moderate and there is a decrease in the proportion of severe uses.
In 2021 there were three authorised projects, at three different sites.

Project 1: 29 uses (average of uses remains constant for this purpose over the last three years). The alternative method is in the process of being validated.

Project 2: 76 uses (the number of uses decreased by more than 50% in 2021 compared to the average uses from 2018 to 2020). The reason for the uses is that not only endotoxins but all pyrogenic agents are evaluated.

Project 3: 6 950 uses, a decrease of almost 15% compared to the average uses in the period 2018-2020. The reason why no alternative methods are used is that 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. The release of the product by this test alone entails the risk of potential pyrogenic substances not due to endotoxins. The activated monocytes test is not recommended as a substitute for the rabbit test given the lack of evidence that pyrogenic agents other than bacterial endotoxins can be detected by all sample categories.

- **Eye irritation/corrosion test in rabbits**

In 2021, nine uses were carried out for this purpose, all authorised in the same project and conducted at the same site, representing a decrease of more than 40% compared to the average for the period 2018-2020.
All uses have been of moderate severity.
The reason why alternative methods are not exclusively used is that after considering existing information on the physicochemical properties of substances and existing information obtained from in vitro tests, it was not possible to draw a conclusion with sufficient confidence on the classification of the substance under the UN GHS (Globally Harmonised System of Classification and Labelling of Chemicals). Therefore, point 28 of 'OECD Guidance Document No 263 on Integrated Approaches to Testing and Assessment (IATA) for Serious Eye Damage and Eye Irritation', which provides for in vivo studies in these cases, is considered applicable.

- **Skin irritation/corrosion test in rabbits**

In 2021, 131 uses were carried out for this purpose, included in three projects. This figure indicates an almost 6% decrease compared to the average for the period 2018-2020.
58% of these uses were mild and the rest moderate.

- Project 1: 76 uses. This figure indicates a 7% increase compared to the average for the period 2018-2020. 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 addressed in a biological risk assessment) of ISO Standard 10993-1 (Biological evaluation of medical devices). The test is performed according to the 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 assess '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 used in this project.

- Project 2: 42 uses. This figure indicates a decrease of more than 20% compared to the average for the period 2018-2020. The reason for these uses is that the in vitro test is validated only for pure chemicals, and not for medical and pharmaceutical extracts. ISO 10993-10 specifies

that for medical devices the in vitro test is performed first, and if the product is not corrosive, the result should be confirmed by carrying out an in vivo test. The in vitro test is also not valid for repeated dose irritation tests.

- Project 3: 13 uses. This figure indicates an almost 70% decrease compared to 2020. The reason why alternative methods are not exclusively used is the need to distinguish Category 3 within the UN GHS system, as detailed in point 92 of the 'New Guidance Document on an Integrated Approach on Testing and Assessment (IATA) for skin corrosion and irritation No 203' (OECD).

- **Skin irritation/corrosion test in rats**

In 2021, 112 uses were carried out for this purpose, included in only one project. This represents a decrease of more than 50% compared to 2020, the only year in which uses for this purpose were reported.

All uses have been of moderate severity.

In vivo dermal acute toxicity studies in accordance with OECD Guideline 402 were necessary to comply with the regulatory requirements laid down in Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market. According to the OECD document 'Guidance Document on Considerations for Waiving or Bridging of Mammalian Acute Toxicity Tests No. 237', it is only possible to dispense with these studies if the criteria set out in points 14 to 18 of that Guidance Document are met, namely: (1) the substance is corrosive or severely irritant to the skin according to previous in vivo or in vitro results. (2) they are end-use products for which the design of the product prevents dermal exposure (3) products for which there are no adverse effects by oral toxicity at doses greater than 2 000 mg/kg body weight. (4) if the oral Lethal Dose 50 is less than 300 mg/kg body weight 5) if the Lethal Dose 50 range is between 300 and 2 000 mg/kg live weight and the dermal absorption data indicate low uptake. Therefore, these in vivo dermal acute toxicity studies were carried out as the OECD exemption criteria were not met and there were no alternative methods validated by the OECD and ECVAM for the assessment of acute toxicity dermal route.

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

This scenario has not been reported.

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 reported for 2021 (268,203) compared to 2020 (274,076 uses) but an increase compared to 2019 (260,525 uses). However, the figures are still approximately similar over the past three years.

Re-use

In 2021, 2,044 animals were re-used (less than 1%) compared to the previous years (2020: 3,094, 1% and 2019: 8,234, 3%). This is a decrease at least in relation to 2019.

Creation of new genetically altered line

There was an increase in the use of animals in the creation of new genetically altered lines in 2021 (19,740) compared to 2020 (14,406), but less uses than in 2019 (23,783). Contrary to last year, zebrafish dominate with 14,864 uses (6,735 uses in 2020), but is similar to 2019 (16,739). The use of mice is clearly lower in 2021 (4,876) compared to 2020 and 2019 (7,671 and 7,044). Only zebrafish and mice were used to create new genetically altered lines.

Genetic status

The use of genetically altered animals with a harmful phenotype increased in 2021 to 22,142 uses compared to 2020 (14,419 uses), but showed a decrease compared to 2019 (25,959). The use of genetically altered without a harmful phenotype was less (95,456) than the previous years; 113,621 in 2020 and 106,496 in 2019.

Maintenance of genetically altered lines

There was an increase in the uses for maintenance of genetically altered lines, 1,225 in 2021, compared to 1,071 in 2020 and 976 in 2019. Only mice were used.

Place of birth

Uses with animals born in the Union at an authorised breeder was lower in 2021 (202,718) compared to 2020 (214,381) and 2019 (218,018). However, uses with animals born in the Union but not at an authorised breeder was higher (61,739) in 2021 compared to 2020 (55,003) and 2019 (32,759 uses). 160 of the uses in 2021 were with animals born in the rest of Europe, less than 2020 (200), but similar to 2019 (148). 1,536 uses were with animals born in the rest of the world, similar to 2020 (1,398) and 2019 (1,361).

Non-human Primate Source

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. In 2019, all of the 28 uses on non-human primates were of primates born in Asia.

Species

Mice

For mice, 158,303 uses are reported in 2021, a clear decrease compared to 2020 (176,073) and less than in 2019 (168,194). The main uses in 2021 were under *Basic Research* (118,796) as for 2020 (136,071) and 2019 (127,780). Mice are used for many different purposes, of which only the purposes with the highest usage is listed here. 27,098 uses were reported under *Nervous System*, similar to 2020 (28,048) and 2019 (25,083). 23,484 uses were reported in *Immune System,* less than both 2020 (32,046) and 2019 (31,143). *Cardiovascular Blood and Lymphatic System* uses increased to 23,274 in 2021 compared to 2020 (22,584) and 2019 (21,068). The use in *Oncology* fluctuated slightly over the past years (2021: 21,491, 2020: 20,116 and 2019: 21,719), whereas a clear decrease was seen in *Endocrine System/Metabolism* in 7,621 compared to 2020 (11,603) and 2019 (15,368). *Basic Research* has a similar percentage of all uses of mice in 2021 (75%) compared to both 2020 (77%) and 2019 (76%). The use within *Translational and applied research* is also similar between 2021 (21%), 2020 (20%) and 2019 (22%). The main uses were within *Human cancer*, 9,415 (9926 in 2020, 11,694 in 2019), *Human Endocrine/Metabolism Disorders,* 8,165 (8,287 in 2020, 7855 in 2021), *Human Respiratory Disorders*, 5,636 (4479 in 2020, 3753 in 2019), *Other Human Disorders,* 2,083 (1,298 in 2020, 1,285 in 2019), and *Human Cardiovascular Disorders,* 2,063 (4722 in 2020, 5128 in 2019).

Rats

The use of rats exhibited a decrease to 15,439 in 2021 compared to 2020 (18,385), but was similar compared to 2019 (15,502). For 2021, as for 2020 and 2019, most uses were reported in *Basic Research* (10,125; 12,520 and 11,106) especially about the *Nervous System* (3,610; 4,997 and 3,884 respectively). Another large use in *Basic research* was the purpose *Multisystemic* (1,811) although lower than in 2020 (3,003) and in 2019 (2,127). Other large areas of use were *Cardiovascular Blood and Lymphatic System* (1,713; 845 and 1,011) and *Immune System* (869; 845 and 1,037). Within *Translational and applied research*, the uses reported for 2021 were 3,793; a decrease compared to 2020 (4,975), but similar to the use in 2019 (3,907), the highest use being within *Human Nervous and Mental Disorders* (1,197) a decrease compared to 2020 (1,327) but a clear increase compared to 2019 (382). The use within *Human Cardiovascular Disorders* (829) was similar to 2020 (768), but higher than in 2019 (620). There was a clear decrease in the use within *Human Respiratory Disorders* (513) compared to both 2020 (1,713) and 2019 (1,230). 819 uses were reported under *Toxicity and other safety testing including pharmacology*, an increase compared to 2020 (439) and especially to 2019 (9), all following legislation satisfying EU requirements. The uses were reported under *Pharmaco-dynamics (incl safety pharmacology)* (612, zero uses in 2020 and 2019) and *Kinetics* (207 compared to 109 in 2020 and 9 in 2019). No uses were reported in *Acute and sub-acute* as compared to 330 uses in 2020, all within *Non lethal methods*.

Guinea pigs

687 uses of guinea pigs were reported in 2021, an increase compared to 2020 (409) and 2019 (437). The highest use was within *Basic research* (589), within *Sensory organs* (346) and in *Respiratory*

System (243). Corresponding figures for 2020 and 2019 were for *Sensory organs* 192 and 253, and for *Respiratory System* 103 and 125. For *Translational and applied research*, total figures for 2021, 2020 and 2019 were 86, 113 and 59 respectively. All 86 uses were reported under *Human Respiratory Disorders*, a slight decrease compared to 2020 (100), but a clear increase compared to 2019 (10).

Syrian hamsters
51 uses of Syrian hamsters were reported in 2021 as compared to zero in 2020 and 2019. All uses were in *Basic Research, Respiratory System*.

Other rodents
56 uses of other rodents were reported, a decrease compared to 82 in 2020, and especially compared to 2019 (304). All uses in 2021 were within *Basic Research, Immune System.* In 2020, the uses were also within *Basic Research*; *Immune System* (79), and in addition *Musculoskeletal System* (3). Main areas of use for 2019 were within *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (219) and *Preservation of species* (169), with the remaining use in *Basic Research* (85); *Ethology/Animal Behaviour/Animal Biology* (50) and *Immune System* (35).

Rabbits
The amount of uses on rabbits (2,798) have decreased compared to 2020 (3,181), but is similar to 2019 (2,765). Most uses are 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 and 2019, they were mostly reported as *Basic Research*, the majority divided between *Other basic research* (2020: 2,057 and 2019: 1,247), *Respiratory System* (2020: 579 and 2019: 662), *Nervous System* (2020: 55 and 2019: 614), and *Cardiovascular Blood and Lymphatic System* (2020: 318 and 2019: 25). 18 uses were reported under *Translational and applied research*, all of them *Human Musculoskeletal Disorders*, similar to 2020 (18) and 2019 (16).

Cats
There were 65 uses of cats in 2021, a decrease compared to 147 uses of cats in 2020 and 288 uses in 2019. All uses 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). In 2019, 262 uses were reported under the same category.

Dogs
There was an increase of the use of dogs in 2021 (566 uses) compared to 2020 (494 uses) and 2019 (364 uses). The majority of uses were within *Basic research* (481) with lower uses in *Translational research* (32) and in *Training for the acquisition, maintenance or improvement of vocational skills* (48). For 2020, 392 uses were in *Translational research*, mostly within *Animal Diseases and disorders* (282 uses), and 102 uses were in *Basic research*, all but two in *Musculoskeletal System*. In 2019, 299 uses were recorded in *Translational research*, mainly in *Animal Diseases and Disorders* (216), 28 in Basic research and 37 in *Higher education or training for the acquisition, maintenance or improvement of vocational skills*.

Ferrets
2 uses of ferrets were reported in 2021 compared to 18 uses in 2020 and 25 uses in 2019. 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* and in 2019 within *Basic Research, Nervous System.*

Other carnivores

215 of the uses of carnivores were reported as other carnivores, a similar figure compared to both 2020 (119) and 2019 (115). 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*, whereas uses in 2019 were reported as *Preservation of Species* (2), *Basic Research* (48) and *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (65).

Horses, donkeys and cross-breeds

Horses, donkeys and cross-breeds had 416 uses in 2021 compared to 570 uses in 2020 and 340 in 2019. Most uses are in *Basic research* (183), mostly in *Musculoskeletal System* (134) and *Multisystemic* (34). In 2020, 198 uses were reported in *Basic Research* (2019: 21). Within *Translational and applied research* 54 uses were reported in 2021, a clear decrease compared to 250 in 2020 and 193 in 2019. 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: 111 uses in *Training for the acquisition, maintenance or improvement of vocational skills* in 2021, 122 uses in 2020 and 126 uses in 2019 under *Higher Education or Training for the Acquisition, Maintenance or Improvement of Vocational Skills*.

Pigs

1,403 uses of pigs were reported in 2021, similar to 2020 (1,325) but less than 2019 (1,730). Most uses were within *Training for the acquisition, maintenance or improvement of vocational skills* (759) comparable to 2020 (689) and 2019 (780), then reported under *Higher education or training for the acquisition, maintenance or improvement of vocational skills*. *Basic Research* follows with 423 uses (373 in 2020 and 664 in 2019), with most uses under *Ethology / Animal Behaviour /Animal Biology* (228). 221 uses were reported under T*ranslational and applied research*, similar to 2020 (263) and 2019 (286). Most uses were in *Animal Nutrition* (80) and *Human Cardiovascular Disorders* (59).

Goats

The use of goats (51) is similar to 2020 (59) and 2019 (53). The main use in 2021 was in *Regulatory use and Routine production, Blood based products*, 34, comparable to 2020 (25). No uses were reported under that purpose in 2019. 17 uses were reported under *Training for the acquisition, maintenance or improvement of vocational skills*, very similar to 16 uses in both 2020 and 2019, then reported under *Higher education or training for the acquisition, maintenance or improvement of vocational skills*. No uses were reported under *Translational and applied research* (13 in 2020 and 25 in 2019).

Sheep

The amount of uses on sheep increased to 232 in 2021 compared to 167 in 2020, but still less than the drastic increase from previous years that was reported in 2019 (442). 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 in 2021 under *Training for the acquisition, maintenance or improvement of vocational skills*, an increase compared to 2020 (20) but

similar to 2019 (47), then recorded under *Higher education or training for the acquisition, maintenance or improvement of vocational skills*. The majority of uses in both 2020 and 2019 were reported in *Translational and applied research* (95 and 395 uses), mostly under *Animal Welfare* (296 in 2019, 0 uses in 2020). In 2020, 52 uses were reported under *Basic Research* (0 uses in 2019).

Cattle

Cattle had 3,383 uses in 2021, which is an increase compared to 2020 (2,908) and 2019 (1,621). Most uses of cattle are recorded in *Training for the acquisition, maintenance or improvement of vocational skills* (1,430) with corresponding figures in 2020 (1,397) and 2019 (1,489), then recorded under *Higher education or training for the acquisition, maintenance or improvement of vocational skills.* There was an increase in Basic research (1,281) compared to 2020 (152) and 2019 (122). 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* (122 in 2019) and 84 under *Other Basic research* (zero in 2019), more specifically within development of feeding or husbandry systems and tests of milking machines. 1,115 uses were reported under *Translational and applied research,* a slight decrease compared to 2020 (1,359), but a clear increase compared to 2019 (10). In 2021, the major part of the animals use in *Translational and Applied Research* was in *Animal Diseases and Disorders* (852) as it was for 2020 (1,116) whereas no uses were reported in this category for 2019. 12 uses in 2021 were reported under *Routine production, Blood based products,* whereas zero use was reported in 2020 and 2019.

Monkeys, Cynomolgus

28 uses of Cynomolgus monkeys were reported in 2021 compared to 4 uses 2020 and 20 in 2019. Most uses (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 and 2019, all uses were in *Basic Research*; in 2020, *Cardiovascular Blood and Lymphatic System* and in 2019, *Nervous System* (17 uses) and *Cardiovascular Blood and Lymphatic System* (3 uses).

Monkeys, Rhesus

31 uses of Rhesus monkeys were reported in 2021 compared to 19 uses in 2020 and 8 uses in 2019. All uses in 2021 were reported as *Translational and Applied Research, Human Immune Disorders*. All uses in 2020 were reported as *Translational and Applied Research, Human Infectious Disorders,* whereas 6 uses in 2019 were reported as *Basic Research, Nervous System* and 2 uses in 2019 were reported as *Translational and Applied Research, Human Infectious Disorders*.

Other mammals

Other mammals had 324 uses in 2021, similar to 382 uses in 2020, but an increase compared to 2019 (200). 171 uses were reported under *Basic Research*, mainly under *Ethology/Animal Behaviour/Animal Biology* (160) but also under *Other basic research* (11) more specifically studies of population structure. 143 uses were reported under *Protection of the natural environment in the interests of the health or welfare of human beings or animals* and 10 under *Regulatory use and Routine production, Blood based products*. The main uses in 2020 and 2019 were in *Basic research* (377, 193). For 2020, most uses were within the category *Ethology/Animal Behaviour/Animal Biology* (301), 76 uses were recorded as virology studies under *Other Basic Research,* and 5 uses under *Routine production, Blood based products*. In 2019, 193 uses were under *Basic Research, Ethology/Animal Behaviour/Animal Biology* and 7 under *Translational and applied research, Animal*

Diseases and Disorders.

Domestic fowl

The use of domestic fowl, 1,182, is lower than in both 2020 (1,988) and 2019 (1,994). 555 uses were reported under *Translational and applied research*, an increase compared to 2020 (190) but lower than 2019 (972). Also, 555 uses were reported under *Basic research*, a decrease compared to both 2020 (1,777) and 2019 (1,022). Main use under *Basic research* was within *Ethology/Animal Behaviour/Animal Biology,* 416, lower than both 2020 (880) and 2019 (826) and the remaining use (139) was in *Multisystemic* (266 in 2020 and 168 in 2019). Zero uses were reported under *Oncology* in neither 2021 nor in 2019, compared to 600 uses in 2020. All uses (555) within *Translational and applied research* were reported under *Animal Diseases and Disorders,* an increase compared to 2020 (146), but lower than in 2019 (718). 72 uses were reported under *Regulatory use and Routine production* (21 in 2020, zero in 2019), all within *Blood based products*.

Other birds

In 2021, 5,526 uses of *Other birds* were reported. This is an increase compared to 2020, (3,906), but a significant decrease compared to 2019 (10,810).

Most uses of *Other birds* were reported under *Basic research* (5,298, of which 5,123 were specified as *Ethology/Animal Behaviour/Animal Biology,* 167 under *Endocrine System/Metabolism* and 8 under *Cardiovascular Blood and Lymphatic System*). 221 uses were reported under *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, and 7 under *Preservation of Species*.

In 2020 and 2019 most uses of *Other birds* were reported under *Basic research* (3,592 and 9,679), of which 3,524 in 2020 and all uses in 2019 were in *Ethology/Animal Behaviour/Animal Biology,* and remaining in 2020 under *Endocrine System/Metabolism* (52) and *Cardiovascular Blood and Lymphatic System* (16). 314 and 1,131 uses were reported under *Protection of the natural environment in the interests of the health or welfare of human beings or animals* for 2020 and 2019, respectively.

Reptiles

No reptiles were reported in 2021. In 2020, there was a decrease to 34 uses compared to 139 uses in 2019. All uses in 2020 were reported as *Basic Research, Ethology/Animal Behaviour/Animal Biology*, whereas the uses in 2019 were reported as *Basic Research, Endocrine System/Metabolism* (110) and *Ethology/Animal Behaviour/Animal Biology* (29).

Frogs, Rana

0 uses of the frogs *Rana temporaria* and *Rana pipiens* were reported in 2021, 2020 and 2019.

Frogs, Xenopus

Xenopus frogs have decreased (93 uses) compared to especially 2020 (1,761) but also to 2019 (171). The majority (69 uses) are reported as *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (2020: 1,679, 2019: 137), and the remaining are uses within *Basic research, Other basic research.* In 2020, a smaller portion was reported as *Basic Research, Nervous System* (58 uses) and *Ethology/Animal behaviour/Animal Biology* (24 uses). In 2019, the use in *Basic Research* was for *Nervous System* only (34 uses).

Other amphibians

The use of *Other amphibians* increased in 2021 (1,871) compared to 2020 (1,256), but showed a

decrease compared to 2019 (2,041). All uses were reported under *Basic research* for 2021, 2020 and 2019, most uses under *Nervous System* (1,264; 1,014 and 897 respectively). 607 uses were reported under *Cardiovascular Blood and Lymphatic System* (242 under 2020 and 298 under 2019).

Zebrafish

Zebrafish uses have increased in 2021 (22,924) compared to 2020 (18,285) but shows a decrease compared to 2019 (35,089). In 2021, 22,453 uses were reported in *Basic research* (16,733 in 2020 and 28,611 in 2019), 471 in *Translational and applied research* (1,552 in 2020 and 6,478 in 2019). The main uses within *Basic Research* are in subcategories *Cardiovascular Blood and Lymphatic System* (2021: 14,404, 2020: 4,644, 2019: 16,103), *Nervous System* (2021: 2,162, 2020: 3,617, 2019: 4,066), *Multisystemic* (2021: 2,017, 2020: 2,663, 2019: 5,378) and *Developmental biology* (2021: 1672, zero uses in 2020 and 2019). Under *Translational and applied research* 251 uses were reported as *Human Nervous and Mental Disorders,* a decrease compared to 2020 (1,432) and especially to 2019 (4,426), and 220 uses were reported in *Non-regulatory toxicology and ecotoxicology*, a tenfold less than in 2019 (2,052). No such use was reported in 2020.

Salmon, trout, chars and graylings

8,881 uses were reported under *Salmon, trout, chars and graylings.* This is the first year these species are being 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 (7,266), followed by Basic Research (1,007), Translational and applied research* (912) and finally *Higher education* (114). Main uses under *Basic Research* was reported in *Ethology/Animal Behaviour/Animal Biology* (593) and in *Cardiovascular Blood and Lymphatic System* (184). Main uses under *Translational and applied research* was under *Animal Nutrition* (504) and *Non-regulatory toxicology and ecotoxicology* (270).

Other fish

The use of *Other fish* is similar in 2021 (42,803) and 2020 (42,504), but more than doubled compared to 2019 (17,873). The major use of *Other fish* is reported under *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (32,807), a large increase compared to 2020 (8,208) and 2019 (2,905), followed by *Translational and Applied Research* (5,930) with similar use as for 2020 (5,883) and 2019 (6,083). Within *Translational and Applied Research*, all uses in 2021 were under *Non-regulatory toxicology and ecotoxicology*. The use in *Basic Research* (3,193) decreased to approximately one-eighth of the use in 2020 (25,709) and half of the use in 2019 (6,010). Within *Basic research,* the uses were in *Ethology/Animal Behaviour/Animal Biology* (2,026) and *Other research* (1,167), more specifically to understand how cyanobacteria blooms affect fish, in particular the herring. The remaining uses were within *Higher education* (747) and *Preservation of species* (126).

In 2020, most uses of *Other fish* are reported under *Basic Research* (25,709 uses, of which 25,005 were in *Ethology/Animal Behaviour/Animal Biology*), *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (8,208) and *Translational and Applied Research* (5,883 uses, all in *Non-regulatory Toxicology and Ecotoxicology*). In 2019, most uses of *Other fish* were reported as *Basic Research* (6,010 uses, of which 5,331 were in *Ethology/Animal Behaviour/Animal Biology*) and *Translational and Applied Research* (6,083 uses, of which 5,619 were in *Non-regulatory Toxicology and Ecotoxicology*). Also, 284 uses were reported under *Animal Diseases and Disorders* and 180 under *Animal Welfare.*

(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.

Basic Research has decreased to 167,087 uses (62%) compared to 202,547 (74%) in 2020, and 190,709 uses (73%) in 2019.

Most uses in *Basic Research* for 2021 were reported under *Cardiovascular Blood and Lymphatic System*, 40,190, an increase compared to 2020 (28,987), but similar to 2019 (38,876). The second largest use was reported under *Nervous System*, 34,322, lower than 2020, (37,926), but again similar to 2019 (34,646). 24,737 uses were reported under *Immune System* (32,975 in 2020, 32,300 in 2019), an increase, which is seen also in *Oncology*, 22,112, (20,806 in 2020, 21,733 in 2019. An increase is seen in *Sensory Organs (skin, eyes and ears)*, 1,245, compared to 2020 (833), but almost half of the uses in 2019 (2,206).

A very large decrease is seen in *Ethology/Animal Behaviour/Animal Biology* 9,838, compared to 30,081 in 2020 and 17,858 in 2019. *Musculoskeletal System* decreased (4,798 uses) compared to 2020 (7,048), but increased in comparison to 2019 (3,960). The use in *Multisystemic* decreased to 7,153 (11,308 in 2020, 9,689 in 2019). 3,360 uses were reported in *Developmental Biology*, which is a new category. A decline is visible in *Other basic research* in 2021, 5,010 uses (3%), compared to 2020 (10,193, 5%) but similar to 2019 (4,763, 2%). Decreases were also shown (1,827 uses) in *Gastrointestinal System including Liver* compared to 2020 (4,341) but not to 2019 (1,772), in the use for *Urogenital/Reproductive System* (1,067, compared to 1,289 in 2020 and 1,967 in 2019). A large decrease is seen for uses in *Endocrine System/Metabolism* (9,085) compared to 2020 (14,163) in 2020 and 2019 (17,894). The use for *Respiratory System* is lower in 2021 (2,361) than in 2020 (2,597) and in 2019 (3,045).

The number of uses in 2021 reported as *Translational and Applied Research*, 46,776 (17%), is proportionally similar to compared to 2020 (50,375, 18%), but slightly lower than 2019 (55,756, 21%).

The largest group of use is *Human Cancer*, 9,420 uses, although it is a decrease in actual numbers compared both to 2020 (10,046) and to 2019 (12,124). The second largest group of use is *Human Endocrine/Metabolism Disorders*, where similar uses were reported for 2021 (8,540), 2020 (8,575) and 2019 (8,331). The third largest group is *Non-regulatory toxicology and ecotoxicology*, with similar use during 2021 (6,622) and 2020 (6,462), but lower than in 2019 (8,423), followed by *Human Respiratory Disorders*, again similar use in 2021 (6279) and 2020, but higher than in 2019 (5,065).

There have been less uses for *Human Infectious Disorders* in 2021 (1,151) compared to 2020 (1,527), but similar to 2019 (1,056). There is a continued decrease in use for *Human Nervous and Mental Disorders* (3,035 in 2021, 5,038 in 2020 and 8,067 in 2019) as well as for *Human Cardiovascular Disorders* (2,954 in 2021, 5,613 in 2020 and 5,890 in 2019). Also, the use in *Diagnosis of Diseases* continues to decrease (15 in 2021, 421 in 2020 and 500 in 2019).

The use in *Human Immune Disorders*, is similar in 2021 (1,182) and 2020 (1,106), but lower than in 2019 (1,910). Approximately the same is seen in *Animal Welfare* where the use is similar (267) to 2020 (247), but less than half of the uses in 2019 (611).

Human Musculoskeletal Disorders decreased in 2020 (101) compared to 2020 (273) but was slightly higher than in 2019 (75).

A clear increase was seen in *Human Gastrointestinal Disorders including Liver* with 678 uses in 2021, compared to 78 in 2020 and zero uses in 2019. Also for *Human Urogenital/Reproductive Disorders*, an increase is seen the last years, 467 (2021), compared to 157 in 2020 and 99 in 2019. *Human Sensory Organ Disorders (skin, eyes and ears)* has increased in relation to prior years (1,199 in 2021, 971 in 2020, 250 in 2019), as have *Other Human Disorders* (2,618 in 2021, 1,616 in 2020 and,559 in 2019).

This is the first year uses could be recorded under *Animal Nutrition*, and 806 uses were reported. *Animal Diseases and Disorders* was lower (1,442) than both 2020 (1,826) and 2019 (1,796).

There is a more than doubled increase in *Regulatory use and Routine production*, 5,399 uses for 2021 compared to 2,080 uses for 2020 and 1,079 in 2019. The main difference is found in in *Routine production* with 391 uses in *Blood based products* and 2,329 uses in *Monoclonal and polyclonal antibodies (excluding ascites)* compared to 2020 when 137 uses were reported in *Blood based products.* In 2019, no animals were used in *Routine production*. A decrease was seen in *Quality control (incl. batch safety and potency testing);* 313 in 2020, compared to 547 in 2020 and 1,070 in 2019, all used in *batch potency testing*. A clear increase was seen in *Toxicity and other safety testing including pharmacology* (2,366, compared to 1,353 in 2020 and 9 in 2019). The main part of this was 1,448 uses in *Pharmacodynamics* and 575 uses in *Carcinogenity*. Zero uses were reported under *Other efficacy and tolerance testing*, 43 in 2020 and again zero uses in 2019.

There is a large increase in *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, 40,540 uses in 2021 compared to 2020 (10,201) and 2019 (4,457).

The category *Preservation of species* has continued to decrease, 221 in 2021 compared to 1,834 in 2020 and 2,416 uses in 2019.

This is the first year that the educational purpose is divided into *Higher education* (2,014 uses) and *Training for the acquisition, maintenance or improvement of vocational skills* (4,914) instead of being reported under the same category *Higher education or training for the acquisition, maintenance or improvement of vocational skills.* Together, they show an increase compared to 2020 (5,986) and 2019 (5,132).

Maintenance of colonies of established genetically altered animals, not used in other procedures had 1,225 uses in 2021, higher than in 2020 (1,071) and in 2019 (976).

There is a continued decrease in *Legislation on medicinal products for veterinary use and their residues*, 313 for 2021 compared to 2020 (547) and 2019 (1,070). There is, on the other hand, a continued increase in the category *Legislation on medicinal products for human use*, in 2020 (2,366) compared to 2020 (1,396) and 2019 (9). 27 uses (all mice) were reported under *Medical devices legislation* compared to zero uses in 2020 and 2019.

It is not clear what the changes depend on, but it is possible that some changes are still due to the effect of Covid-19, concerning both research not performed according to plan as well as research that was initiated due to the disease.

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

Severities

The actual severities were proportionally similar during the past years for *Non-recovery* 2021 (9,390, 4%), 2020 (9,175, 3%), and 2019 (9,370, 4%). The proportion for *Mild* has increased in 2021 (88,169, 33%) compared to 2020 (69,065, 25%), but is similar to 2019 (79,396, 30%). *Moderate* severity has decreased in 2021 (149,411, 56%), compared to 2020 (172,068, 63%), but is similar to 2019 (144,917, 56%). *Severe* is similar in 2021 (21,085, 8%) compared to 2020 (23,768, 9%), 2019 (26,842, 10%).

Mild

Most uses classified as *Mild* are reported as *Basic Research* during the past years, but the ratio is decreasing (2021: 63,845, 73%, 2020: 53,757, 78%, and 2019: 69,540, 88%). There is a continued large increase in the use under *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, in 2021, (13,478, 15%) compared to previous years (2020: 6,611, 10%, and 2019: 469, less than 1%). Uses with in education is proportionally the same during the past three years, 3% (2021: 2,602, 2020: 2,042, and 2019: 2,399), although it is now reported in two categories instead of one (*Higher education* and *Training for the acquisition, maintenance or improvement of vocational skills).* The use classified as Mild category in *Creation of new genetically altered animal lines* increased to 86,994 (16%) compared to 2020 (68,598, 9%), but showed a decrease compared to 2019 (48,896, 22%). *Maintenance of colonies of established genetically altered animals, not used in other procedures* has 1,175 uses in Mild to be compared with 467 in 2020 and 500 in 2019.

Moderate

Most uses classified as *Moderate* are reported as *Basic Research* during the past years, although decreasing in 2021 (77,376, 52%) compared to 2020 (121,113, 71%) and 2019 (93,666, 65%). The decrease is spread over several areas, but the largest decrease is within *Ethology/Animal Behaviour/Animal Biology* (2021: 2,920, 2020: 26,442 and 2019: 8,042). Also, the use in *Gastrointestinal System including Liver* has decreased to 1,203 in 2021 from 3,723 in 2020, although a slight increase compared to 2019 (893). Decreases are also shown in *Immune System*, 12,557 compared to 16,831 in 2020 and 14,101 in 2019, and in *Multisystemic* (2021:1,536, 2020: 5,350 and 2019: 1,149). *Translational and applied research* remains at about half of the use in *Basic Research* (2021: 36,410, 2020: 39,796, and 2019: 40,823) and is also decreasing in several areas. Proportionally, the largest decreases compared to 2020 is shown in *Human Cardiovascular Disorders* (2021: 1,681, 2020: 3,769 and 2019: 2,873), and in *Human Nervous and Mental Disorders* (2021: 2,208, 2020: 4,384 and 2019: 7,013).

Severe

Most uses classified as *Severe* in 2021 were reported in *Basic Research* (19,132, 90%), as for the previous year 2020 (20,845, 88%) and 20,404 (76%) and are showing an increase.

The largest increase in found in *Musculoskeletal System* with 1,918 uses (10%) compared to 846 (4%) in 2020 and 535 (3%) in 2019. The use in *Translational and applied research* has decreased in 2021 to 1,791 (8%) from 2,701 (11%) in 2020 and 6,437 (24%) in 2019. The main decrease is shown in *Human Cancer*, 193 (10%) compared to 898 (33%) in 2020 and 3,390 (53%) in 2019, and in *Human Cardiovascular Disorders*, 80 (4%) in 2020 compared to 372 (14%) in 2020 and 375 (6%) in 2019. The use in *Regulatory use and Routine production* has increased slightly to 310 (1%) compared to 66 (less than 1%) in 2020 and zero use in 2019. No uses were reported neither in 2021 nor in 2019 for educational purposes, compared to 156 uses in 2020. 148 uses are reported as *Severe* in the *Creation of New Genetically Altered Line* compared to zero uses for 2020 and 2019.

Non-recovery

Most uses classified as *Non-recovery* were reported as *Basic Research* during the past years. A slight decrease is shown in 2021 (6,659, 71%), compared to both 2020 (6,719, 73%) and 2019 (7,099, 76%). In *Basic Research*, the main uses are reported under *Nervous System*, 2,643 (39%), an increase compared to both 2020 (1,388, 20%) and 2019 (1,872, 26%). Decreases are shown in the uses in *Respiratory System*, 481 (7%) in 2021, compared with 1,322 (19%) in 2020 and 833 (12%) in 2019, as well as in *Immune System* that had 713 (11%) uses compared to 1,671 (25%) in 2020 and 1.482 (21%) uses in 2019. Continued decrease is also shown in *Urogenital/Reproductive System* with zero uses in 2021, 202 (3%) in 2020 and 562 (8%) in 2019) and *Endocrine System/Metabolism* with 382 (6%) in 2021, 535 (8%) in 2020 and 840 (12%) in 2019.

Translational and applied research remains lower than the use in *Basic Research*, 1,757 (19%) in 2021, 1,769 (19%) in 2020 and 2,014 (21%) in 2019, with most uses under *Human Cardiovascular Disorders* (2021: 717, 2020: 788 and 2019: 712). 91 uses were reported under *Regulatory use and Routine production* (1%) whereas zero uses were recorded for 2020 and 2019. Uses within education is increasing (2021: 808, 9%, 2020: 574, 6% and 2019: 2,399, 3%). This use is now reported in two categories instead of one, *Higher education* (298) and *Training for the acquisition, maintenance or improvement of vocational skills* (510).

It is unclear what the changes depend on in this part. However, minor fluctuations are commonly observed over the years.

(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.

Webinars, workshops and meetings

Thanks to the rapid move into digital meetings due to the pandemic years, the National Committee and the 3Rs Center has continued to promote the 3Rs through webinars and digital workshops or other meetings. They arrange short webinars almost every month within all 3 Rs. Some webinars have 30 attendees, others have had more than 100 attendees. Some of the webinars are held in Swedish, some of them in English.

One of the Center's most popular events is a Refinement workshop that they have organised six times during 2021. Together with colleagues from RISE, Research Institutes of Sweden, and the Swedish University of Agricultural Sciences, they demonstrate and discuss refinement techniques and enrichment for mice and rats.

The Swedish 3Rs Center and the National Committee also participate in events organised by others. On several occasions the Center has had the opportunity to present their work – as part of the program or as a poster.

Networking and collaboration

In order to draw attention to the progress in European 3Rs work, the Center together with five other 3Rs centres in Europe, arranged four webinars about replacement of animal-derived products, for example to support cell growth or differentiation.

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 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 and a replace strategy have proceeded – with an open- as well as several internal workshops. The strategy will be formed together with all interested parties and stakeholders. This work will continue in 2022 and 2023.

The 3Rs Center also continues its collaboration with Swedish government agencies to support their 3Rs work. They organise seminars and other meetings together. The 3Rs Center support 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 completed in 2021 to be 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 2021, four editions were published: on themes such as Refinement and rodents, Replacement and computer models, Toxicology, and finally Wildlife and the 3Rs.

Besides 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.

During 2021, the Center released its first films about replacing animal experimentation, with a focus on computer models. The Center has also produced various infographics that in a clear and

pedagogical way show what researchers should keep in mind when to develop and evaluate a method, when formal validation is needed and when a simpler evaluation is sufficient.

It is difficult to measure whether the actions that the Center performs have reduced the number of animals used in Swedish research. However, the Committee and the 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:

Other carnivores
215 of the uses of carnivores were recorded as other carnivores (25%), an increase compared with 119 in 2020 and 115 in 2019. Individual species are brown bear (*Ursus arctos*, 74), American mink (*Neovison vison*, 72), raccoon dog (*Nyctereutes procyonoides*, 29), European polecat (*Mustela putorius*, 26), wolverine (*Gulo gulo*, 11), lynx (*Lynx lynx*, 2), and wolf (*Canis lupus*, 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*. In 2019, brown bear (65), raccoon dog (39), wolf (6), American mink (2*)* and lynx (3), and were reported as *Preservation of Species*, *Basic Research* with subcategory *Ethology/Animal Behaviour/Animal Biology* and *Protection of the Natural Environment in the Interests of the Health or Welfare of Human Beings or Animals*.

Other birds
5,526 (82%) of the birds were reported as *Other birds* in 2021, an increase compared with 2020 (3,906, 66%) and much lower actual number than in 2019, but similar proportionally (10,810, 84%).

The largest uses are found in great tit (*Parus major*, 1,300), European robin (*Erithacus rubecula*, 433), zebra finch (*Taeniopygia guttata*, 372), European pied flycatcher (*Ficedula hypoleuca*, 397), willow warbler (*Phylloscopus trochilus*, 363), Eurasian blue tit (*Cyanistes caeruleus*, 292), great reed warbler (*Acrocephalus arundinaceus*, 221), common blackbird (*Turdus merula*, 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 uses), zebra finch (340), Eurasian blue tit (265), common blackbird (235) and the great reed warbler (211). In 2019, main uses were with collared flycatcher (*Ficedula albicollis*, 3,263), great tit (3,161) and Eurasian blue tit (1,966 uses).

Other fish
42,803 (57%) of the reported uses of fish constitutes of *Other fish*, a decrease proportionally speaking to 2020 (42,504, 70%) but a large increase compared to 2019 (17,873, 34%). Most *Other fish* are reported as roach (*Rutilus rutilus*, 10,396), European perch (*Perca fluviatilis*, 6,026), three-spined stickleback (*Gasterosteus aculeatus*, 5,770), common bleak (*Alburnus alburnus*,

5,136), Atlantic herring (*Clupea harengus*, 3,664), minnow (*Phoxinus phoxinus*, 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*, 1606) and guppy (*Poecilia reticulata*, 1,586). In 2019, main uses were with European perch (4,785), Atlantic herring (2,754 uses) and guppy (1,840). Since there now is a separate category for *Salmonidae*, the species recorded under *Other fish* has changed.

Other amphibians
95 % of the amphibian use in 2021 were reported as *Other amphibians*, 1,871. Most of these were Iberian ribbed newt (*Pleurodeles waltl*, 1,846) and the remaining 25 were eastern newt (*Notophthalmus viridescens*). In 2020, 1,230 uses of Iberian ribbed newt and 26 uses of eastern newt were reported compared to 2019 with 1,149 uses of Iberian ribbed newt, 46 uses of eastern newt and 846 uses of moor frog (*Rana arvalis*).

Other mammals
324 uses of *Other mammals* were reported, a very small portion of the mammals (183,253), less than 1%. They were 164 wild boars (*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 boars, 118 elk, 38 roe deer, 34 Daubenton's bats (*Myotis daubentonii*), 33 soprano pipistrelles (*Pipistrellus pygmaeus*), 8 Brandt's bats (*Myotis brandtii*), 5 alpaca, and 1 whiskered bat (*Myotis mystacinus*). In 2019, 200 uses (less than 1%) were reported, 69 elk, 64 roe deer, 51 wild boar, 7 common shrews (*Sorex araneus*), 6 red deer (*Cervus elaphus*), 2 Eurasian pygmy shrews (*Sorex minutus*) and 1 fallow deer (*Dama dama*).

(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:

- There was an overall increase of 40% in the total number of animals between 2020 and 2021. The increase in fish is the major reason, followed by mice and rats.
    - The number of fish used in procedures increased from 1,4 million in 2020 to 1,9 million in 2021. This increase was due to the use of a high number of salmon in two projects on the removal of salmon lice in 2021 (a total of almost 800000), whereas no such projects were reported in 2020.
    - The number of mice used in procedures went from 50 222 in 2020 to 52 554 in 2021. However, the number in 2020 was lower than expected due to the pandemic. In 2019 the number was 54 350.
        - The number of rats went from 3 355 in 2020 to 4 498 in 2021. There is not much variation in the number of approved projects using rats, but high numbers of animals have been reported in 2021 from three specific projects
- There has been a decrease in the use of domestic fowl.

(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 has dropped significantly – this is because we have had a successful correction of previous wrong reporting.
- Preservation of species has increased significantly (1,586%) – this is also because we have had a successful correction of previous wrong reporting. A high proportion of salmonids and especially Atlantic salmon are used for the purpose of preservation of species. In some projects the differentiation between “Preservation of species” and “Protection of the natural environment in the interests og the health or welfare of human beings or animals” is not always clear.
- For future narratives, information on significant increase or decrease in other specific areas of purpose will be presented with analyses of the reasons thereof.

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

The higher proportion of animals used in moderately severe experiments in 2021 compared to 2020 (55,7% vs. 34,2 %) is a result of two projects on development of methods for removing salmon lice from salmon. Among the animals exposed to moderate severity Atlantic salmon used in these two projects alone, represented 71,6 % of the total.

The proportion of animals reported in the “severe”, “mild” and “non recovery” categories were slightly lower than in 2020, but not to the extent that we have been able to give explanations for these changes from 2020 to 2021.

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

- We have an extensive dialogue with the applicant while evaluating the application, often resulting in refinements and sometimes also reductions.
- 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.
- We continue our practice with mandatory use of multimodal analgesia for all surgical procedures on rats and mice. (General anaesthesia, local anaesthesia, pre-emptive and postoperative analgesia, preferably 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.

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

No ‘other’ categories 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:

For vaccines, including fish vaccines, the use of alternative methods for batch testing is recognized under the legislation. To our knowledge batch testing of the IPNV component of fish vaccines is the only recognised test not using live animals, i.e. by measuring the amount of virus. No animals have been used for batch testing of the IPNV component.

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

We have no cases where 'severe' classification is exceeded.

VI. Member State comparative tables for 2021

Introduction

Four Member State comparative tables are provided for 2021 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	146,409	228,732	3,310	31,598	2,817	67,143	136,628	1,563	52,856	1,052,503	1,048,354	19,093	60,582	91,497	271,072
	Rats	3,769	13,471	1,907	3,647	0	16,176	25,126	375	9,835	152,654	130,858	2,352	26,971	17,050	90,142
	Guinea-Pigs	135	7,989	24	29	0	1,971	1,991	0	4	49,847	8,591	0	3,737	774	12,183
	Hamsters (Syrian)	479	4,474	0	0	0	290	239	0	415	11,784	2,845	0	74	32	547
	Hamsters (Chinese)	0	0	0	0	0	40	0	0	0	56	0	0	0	0	0
	Mongolian gerbil	12	6	0	0	0	0	0	0	0	423	2,950	0	0	0	0
	Other rodents	4	120	0	0	0	963	12	0	2,018	1,477	6,074	0	0	19	159
	Rabbits															
	Rabbits	974	61,082	64	42	3	5,005	4,363	48	44	170,214	61,738	76	1,445	657	8,461
	Carnivores															
	Cats	8	235	0	0	5	75	3	0	142	439	419	0	11	13	0
	Dogs	278	485	126	0	14	230	328	0	1,824	2,600	1,388	0	328	83	674
	Ferrets	0	0	0	0	0	136	0	0	0	243	119	0	0	358	29
	Other carnivores	0	0	0	0	0	0	0	0	413	1,428	24	0	0	0	0
	Farm animals															
	Horses, donkeys and cross-breeds	45	310	6	0	0	210	48	0	28	139	1,276	0	12	9	36
	Pigs	2,566	4,887	0	2	0	1,590	17,131	4	444	14,809	10,681	148	3,035	727	2,509
	Goats	0	53	0	0	0	86	8	0	0	388	190	0	0	31	13
	Sheep	111	569	0	18	0	454	25	0	335	4,204	2,671	0	82	2,806	70
	Cattle	1,078	867	0	0	0	737	1,880	254	71	1,308	5,582	0	3	2,180	1,124
	Non-human primates															
	Prosimians	0	0	0	0	0	0	0	0	0	59	0	0	0	0	0
	Marmoset and tamarins	0	0	0	0	0	0	0	0	0	89	79	0	0	0	0
	Cynomolgus monkey	0	0	0	0	0	0	0	0	0	2,555	1,320	0	0	0	529
	Rhesus monkey	0	5	0	0	0	0	0	0	0	65	44	0	0	0	0
	Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
	Baboons	0	0	0	0	0	0	0	0	0	38	6	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	92	46	0	0	0	62	26	0	150	194	2,027	0	0	0	11
Birds																
	Domestic fowl	3,542	54,409	100	1,836	0	21,844	1,534	74	468	77,598	18,081	2,095	39,053	98	35,358
	Turkey	0	2,473	0	0	0	518	0	0	1	13,864	80	0	0	0	54
	Other birds	943	2,763	0	0	0	3,702	467	302	742	10,185	7,572	0	3,788	92	96
Reptiles																
	Reptiles	0	7	0	0	0	230	154	0	0	534	269	0	0	0	0
Amphibians																
	Rana	0	0	160	0	0	0	0	0	0	0	2,632	0	0	0	0
	Xenopus	181	124	0	0	70	0	90	0	0	2,074	7,467	0	0	53	326
	Other amphibians	5,316	109	270	0	0	124	137	0	250	1,866	2,830	0	0	0	0
Fish																
	Zebra fish	9,939	38,718	0	420	0	7,390	8,401	0	7,411	62,627	95,479	2,402	11,355	0	13,555
	Sea bass	0	0	0	0	0	0	2,762	0	0	16,912	954	7,732	0	0	62,799
	Salmon, trout, chars and graylings	1,296	781	0	0	0	7,334	8,761	81	14,006	48,843	23,398	0	360	4,986	2,722
	Guppy, swordtail, molly, platy	0	350	0	0	0	84,479	0	0	0	0	500	0	280	0	490
	Other fish	6,234	8,317	0	0	0	19,349	2,439	1,185	302	64,285	38,552	5,585	465	93	2,344
Cephalopods																
	Cephalopods	0	0	0	0	0	0	0	0	0	1,102	56	0	0	0	46
Totals																
	Total	**183,411**	**431,382**	**5,967**	**37,592**	**2,909**	**240,138**	**212,553**	**3,886**	**91,759**	**1,767,419**	**1,485,109**	**39,483**	**151,581**	**121,558**	**505,349**
	%	**1.9**	**4.6**	**0.1**	**0.4**	**0**	**2.6**	**2.3**	**0**	**1**	**18.8**	**15.8**	**0.4**	**1.6**	**1.3**	**5.4**

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	1,678	2,473	4,114	0	135,011	42,639	57,384	53,579	8,155	4,304	4,383	408,079	151,442	**4,087,398**	**43.5**
Rats	584	998	28	0	93,087	4,486	13,919	5,467	4,748	4,978	73	44,650	15,407	**682,758**	**7.3**
Guinea-Pigs	0	0	0	0	5,865	120	5,378	0	363	315	0	8,824	687	**108,827**	**1.2**
Hamsters (Syrian)	132	0	0	0	4,297	30	4	0	32	0	40	1,901	51	**27,666**	**0.3**
Hamsters (Chinese)	0	0	0	0	0	0	0	0	0	0	0	0	0	**96**	**0**
Mongolian gerbil	0	0	0	0	164	0	86	0	0	22	0	40	0	**3,703**	**0**
Other rodents	0	0	0	0	1,494	601	4,493	50	0	0	0	262	56	**17,802**	**0.2**
Rabbits															
Rabbits	12	288	0	0	19,459	20	664	8	275	96	0	18,554	2,796	**356,388**	**3.8**
Carnivores															
Cats	299	0	0	0	207	0	0	0	0	17	0	83	65	**2,021**	**0**
Dogs	0	0	0	0	628	40	3	0	0	0	0	609	495	**10,133**	**0.1**
Ferrets	0	0	0	0	483	0	0	0	0	0	0	125	2	**1,495**	**0**
Other carnivores	0	0	0	0	38	99	0	0	0	0	0	36	148	**2,186**	**0**
Farm animals															
Horses, donkeys and cross-breeds	0	0	0	0	163	87	166	0	0	0	0	252	326	**3,113**	**0**
Pigs	7	105	0	0	7,553	695	884	247	16	0	24	9,281	847	**78,192**	**0.8**
Goats	0	0	0	0	58	0	65	0	0	0	0	154	40	**1,086**	**0**
Sheep	0	0	0	0	1,996	553	158	21	7	0	40	1,298	232	**15,650**	**0.2**
Cattle	2	0	0	0	2,234	194	265	15	0	0	0	1,263	3,011	**22,068**	**0.2**
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	0	0	0	0	**59**	**0**
Marmoset and tamarins	0	0	0	0	2	0	0	0	0	0	0	0	0	**170**	**0**
Cynomolgus monkey	0	0	0	0	12	0	0	0	0	0	0	325	6	**4,747**	**0.1**
Rhesus monkey	0	0	0	0	163	0	0	0	0	0	0	1	0	**278**	**0**
Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	0	0	0	0	**3**	**0**
Baboons	0	0	0	0	0	0	0	0	0	0	0	0	0	**44**	**0**
Squirrel monkey	0	0	0	0	0	0	0	0	0	0	0	0	0	**13**	**0**
Other mammals															
Other mammals	270	0	0	0	261	648	616	73	0	0	0	66	283	**4,825**	**0.1**
Birds															
Domestic fowl	0	0	0	0	41,416	867	3,598	120	1,236	388	74	108,914	1,182	**413,885**	**4.4**
Turkey	0	0	0	0	76	0	45	0	0	0	0	123	0	**17,234**	**0.2**
Other birds	284	147	0	0	12,278	12,992	4,296	13	26	139	0	3,013	5,519	**69,359**	**0.7**
Reptiles															
Reptiles	0	0	0	0	101	10	0	0	0	0	0	1,128	0	**2,433**	**0**
Amphibians															
Rana	0	0	0	0	0	0	0	0	0	0	0	0	0	**2,792**	**0**
Xenopus	0	0	0	0	2,943	0	0	6,777	0	0	0	349	69	**20,523**	**0.2**
Other amphibians	0	0	0	0	12	500	197	0	0	0	0	3,090	1,871	**16,572**	**0.2**
Fish															
Zebra fish	0	0	825	0	12,843	21,702	10,822	1,223	0	0	0	27,152	8,052	**340,316**	**3.6**
Sea bass	0	0	0	26,943	135	210	0	5,235	0	0	0	522,394	0	**646,076**	**6.9**
Salmon, trout, chars and graylings	0	0	0	40	1,986	1,775,161	1,339	780	0	0	0	15,411	7,075	**1,914,360**	**20.4**
Guppy, swordtail, molly, platy	0	0	0	0	1,688	0	1,000	0	0	0	0	0	0	**88,787**	**0.9**
Other fish	0	1,786	0	21,291	57,187	128,035	3,099	1,444	0	0	55	36,414	41,630	**440,091**	**4.7**
Cephalopods															
Cephalopods	0	0	0	0	343	0	0	0	0	0	0	1,537	0	**3,084**	**0**
Totals															
Total	**3,268**	**5,797**	**4,967**	**48,274**	**404,183**	**1,989,689**	**108,481**	**75,052**	**14,858**	**10,259**	**4,689**	**1,215,328**	**241,292**	**9,406,233**	**100**
%	**0**	**0.1**	**0.1**	**0.5**	**4.3**	**21.2**	**1.2**	**0.8**	**0.2**	**0.1**	**0**	**12.9**	**2.6**	**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	147,278	229,330	3,310	31,694	2,817	68,111	138,005	1,563	52,874	1,062,822	1,074,935	19,093	60,827	91,497	271,272
Rats	3,769	13,690	1,907	3,647	0	16,213	25,552	375	9,845	155,607	133,883	2,352	27,083	17,050	90,870
Guinea-Pigs	135	7,997	24	49	0	1,998	1,991	0	4	50,322	8,731	0	3,951	774	12,269
Hamsters (Syrian)	479	4,474	0	0	0	290	239	0	415	12,224	2,849	0	74	32	564
Hamsters (Chinese)	0	0	0	0	0	40	0	0	0	56	0	0	0	0	0
Mongolian gerbil	12	6	0	0	0	0	0	0	0	423	2,971	0	0	0	0
Other rodents	4	132	0	0	0	963	12	0	2,018	1,505	6,075	0	0	19	159
Rabbits															
Rabbits	1,002	61,104	364	42	3	5,032	4,370	48	44	171,894	62,762	76	1,493	657	8,931
Carnivores															
Cats	8	242	0	0	5	171	3	0	142	1,018	862	12	11	65	0
Dogs	291	1,243	126	0	14	492	637	0	1,918	4,376	2,657	9	408	194	751
Ferrets	0	0	0	0	0	136	0	0	0	251	127	0	0	358	33
Other carnivores	0	0	0	0	0	0	0	0	413	1,428	24	0	0	0	0
Farm animals															
Horses, donkeys and cross-breeds	65	347	6	13	0	224	52	0	74	668	1,852	0	58	19	46
Pigs	2,566	5,186	0	2	0	1,607	17,222	4	448	15,025	10,935	148	3,079	727	2,544
Goats	0	54	0	0	0	95	8	0	0	579	201	0	0	31	33
Sheep	111	589	240	38	0	758	41	0	625	4,587	2,832	0	82	2,809	236
Cattle	1,089	1,044	0	0	0	775	1,898	254	608	1,906	6,067	0	3	2,829	1,124
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	60	4	0	0	0	0
Marmoset and tamarins	0	0	0	0	0	0	0	0	0	97	83	0	0	0	1
Cynomolgus monkey	0	0	0	0	0	0	0	0	0	3,276	1,722	0	0	0	593
Rhesus monkey	0	39	0	0	0	0	0	0	0	76	62	1	8	0	0
Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	3	4	0	0	0	0
Baboons	0	0	0	0	0	0	0	0	0	40	6	0	0	0	0
Squirrel monkey	0	0	0	0	0	0	0	0	0	30	3	0	0	0	0
Other species of Old World Monkeys (Cercopithecoidea)	0	0	0	0	0	0	0	0	0	11	2	0	0	0	0
Other mammals															
Other mammals	92	104	0	0	0	62	28	0	150	218	2,063	0	0	0	51
Birds															
Domestic fowl	3,542	54,511	200	1,836	0	21,901	1,534	74	479	77,598	18,351	2,095	39,055	98	35,415
Turkey	0	2,473	0	0	0	521	0	0	1	13,864	80	0	0	0	54
Other birds	949	2,765	0	0	0	3,744	467	302	742	10,191	7,908	0	3,804	92	183
Reptiles															
Reptiles	0	36	0	0	0	230	154	0	0	839	272	0	0	0	0
Amphibians															
Rana	0	0	160	0	0	0	0	0	0	0	2,632	0	0	0	0
Xenopus	242	212	0	0	70	0	740	0	0	3,809	8,655	0	0	53	354
Other amphibians	5,316	109	270	0	0	124	139	0	250	1,866	2,830	0	0	0	0
Fish															
Zebra fish	9,939	38,718	0	420	0	7,390	8,401	0	7,411	63,268	96,011	2,402	11,355	0	13,555
Sea bass	0	0	0	0	0	0	2,762	0	0	16,912	954	7,732	0	0	62,875
Salmon, trout, chars and graylings	1,296	861	0	0	0	7,334	8,763	81	14,006	48,933	24,038	0	360	4,986	2,722
Guppy, swordtail, molly, platy	0	350	0	0	0	84,857	0	0	0	0	663	0	528	0	490
Other fish	6,234	8,711	0	0	0	19,349	2,439	1,185	302	64,428	39,028	6,308	530	93	2,394
Cephalopods															
Cephalopods	0	0	0	0	0	0	0	0	0	1,134	57	0	0	0	46
Totals															
Total	**184,419**	**434,327**	**6,607**	**37,741**	**2,909**	**242,417**	**215,457**	**3,886**	**92,769**	**1,791,344**	**1,523,191**	**40,228**	**152,709**	**122,383**	**507,565**
%	**1.9**	**4.6**	**0.1**	**0.4**	**0**	**2.5**	**2.3**	**0**	**1**	**18.8**	**16**	**0.4**	**1.6**	**1.3**	**5.3**

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 2)

	Latvia	Lithuania	Luxembourg	Malta	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	Total	%
Mammals															
Rodents															
Mice	1,862	2,478	4,114	0	135,903	42,642	57,414	55,634	8,155	4,304	5,247	409,641	151,689	**4,134,511**	**43.5**
Rats	584	998	28	0	93,614	4,492	13,949	5,467	4,783	4,978	73	44,788	15,439	**691,036**	**7.3**
Guinea-Pigs	0	0	0	0	5,870	120	5,382	0	378	315	0	9,058	687	**110,055**	**1.2**
Hamsters (Syrian)	132	0	0	0	4,312	30	4	0	32	0	40	1,901	51	**28,142**	**0.3**
Hamsters (Chinese)	0	0	0	0	0	0	0	0	0	0	0	0	0	**96**	**0**
Mongolian gerbil	0	0	0	0	164	0	86	0	0	22	0	40	0	**3,724**	**0**
Other rodents	0	0	0	0	1,494	601	4,627	50	0	0	0	262	56	**17,977**	**0.2**
Rabbits															
Rabbits	12	288	0	0	19,508	20	968	8	275	96	68	21,233	2,798	**363,096**	**3.8**
Carnivores															
Cats	299	0	0	0	285	0	0	0	0	17	0	741	65	**3,946**	**0**
Dogs	0	0	0	0	912	41	8	0	0	0	0	1,138	566	**15,781**	**0.2**
Ferrets	0	0	0	0	510	0	0	0	0	0	0	125	2	**1,542**	**0**
Other carnivores	0	0	0	0	38	139	0	0	0	0	0	36	215	**2,293**	**0**
Farm animals															
Horses, donkeys and cross-breeds	0	0	0	0	242	92	177	0	2	0	2	303	416	**4,658**	**0**
Pigs	7	105	0	0	7,933	705	884	272	16	0	24	9,596	1,403	**80,438**	**0.8**
Goats	0	0	0	0	190	404	65	93	0	0	0	159	51	**1,963**	**0**
Sheep	0	0	0	0	2,187	569	178	21	199	0	42	1,692	232	**18,068**	**0.2**
Cattle	2	0	0	0	3,377	294	265	15	2	0	0	2,062	3,838	**27,452**	**0.3**
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	0	0	0	0	**64**	**0**
Marmoset and tamarins	0	0	0	0	2	0	0	0	0	0	0	0	0	**183**	**0**
Cynomolgus monkey	0	0	0	0	13	0	0	0	0	0	0	628	28	**6,260**	**0.1**
Rhesus monkey	0	0	0	0	200	0	0	0	0	0	0	1	31	**418**	**0**
Vervets (Chlorocebus spp.)	0	0	0	0	0	0	0	0	0	0	0	0	0	**7**	**0**
Baboons	0	0	0	0	0	0	0	0	0	0	0	0	0	**46**	**0**
Squirrel monkey	0	0	0	0	0	0	0	0	0	0	0	0	0	**33**	**0**
Other species of Old World Monkeys (Cercopithecoidea)	0	0	0	0	0	0	0	0	0	0	0	0	0	**13**	**0**
Other mammals															
Other mammals	270	0	0	0	263	678	616	73	0	0	0	111	324	**5,103**	**0.1**
Birds															
Domestic fowl	0	0	0	0	41,605	867	3,616	120	1,290	388	266	108,996	1,182	**415,019**	**4.4**
Turkey	0	0	0	0	88	0	45	0	3	0	0	123	0	**17,252**	**0.2**
Other birds	284	147	0	0	12,448	12,992	4,312	13	26	139	0	3,582	5,526	**70,616**	**0.7**
Reptiles															
Reptiles	0	0	0	0	101	10	0	0	0	0	0	1,158	0	**2,800**	**0**
Amphibians															
Rana	0	0	0	0	0	0	0	0	0	0	0	0	0	**2,792**	**0**
Xenopus	0	0	0	0	2,943	0	0	6,777	0	0	0	354	93	**24,302**	**0.3**
Other amphibians	0	0	0	0	12	500	197	0	0	0	0	3,123	1,871	**16,607**	**0.2**
Fish															
Zebra fish	0	0	825	0	12,843	21,914	10,822	1,223	0	0	0	27,392	8,060	**341,949**	**3.6**
Sea bass	0	0	0	27,023	135	210	0	5,754	0	0	0	526,820	0	**651,177**	**6.8**
Salmon, trout, chars and graylings	0	0	0	170	1,986	1,775,172	1,339	780	0	0	0	15,411	7,083	**1,915,321**	**20.1**
Guppy, swordtail, molly, platy	0	0	0	0	1,688	0	1,000	0	0	0	0	0	0	**89,576**	**0.9**
Other fish	0	1,786	0	21,443	57,187	128,055	3,099	1,535	0	0	55	39,990	41,630	**445,781**	**4.7**
Cephalopods															
Cephalopods	0	0	0	0	343	0	0	0	0	0	0	1,537	0	**3,117**	**0**
Totals															
Total	**3,452**	**5,802**	**4,967**	**48,636**	**408,396**	**1,990,547**	**109,053**	**77,835**	**15,161**	**10,259**	**5,817**	**1,232,001**	**243,336**	**9,513,214**	**100**
%	**0**	**0.1**	**0.1**	**0.5**	**4.3**	**20.9**	**1.1**	**0.8**	**0.2**	**0.1**	**0.1**	**13**	**2.6**	**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	Romania	Spain	Sweden	Total	%
Mice	No	13,040	23,785	0	8,074	7,503	11,348	25,660	130,283	511	141	60	3,620	289	0	3,808	1,889	421	474	0	18,630	4,876	**254,412**	**99.4**
Mice	Yes	0	5	0	0	21	0	658	277	0	0	0	23	0	0	0	0	0	0	0	582	0	**1,566**	**0.6**
Mice	**Total**	**13,040**	**23,790**	**0**	**8,074**	**7,524**	**11,348**	**26,318**	**130,560**	**511**	**141**	**60**	**3,643**	**289**	**0**	**3,808**	**1,889**	**421**	**474**	**0**	**19,212**	**4,876**	**255,978**	**100.0**
Rats	No	0	133	0	112	0	0	1,441	29	0	0	0	133	0	0	0	0	0	0	90	0	0	**1,938**	**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**	**0.0**
Rats	**Total**	**0**	**133**	**0**	**112**	**0**	**0**	**1,441**	**29**	**0**	**0**	**0**	**133**	**0**	**0**	**0**	**0**	**0**	**0**	**90**	**0**	**0**	**1,938**	**100.0**
Hamsters (Syrian)	No	0	270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**270**	**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**	**0.0**
Hamsters (Syrian)	**Total**	**0**	**270**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**270**	**100.0**
Rabbits	No	0	0	0	0	0	0	326	9	0	36	0	0	0	0	0	0	0	0	0	20	0	**391**	**99.7**
Rabbits	Yes	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**0.3**
Rabbits	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**327**	**9**	**0**	**36**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**20**	**0**	**392**	**100.0**
Pigs	No	0	0	0	62	0	0	9	208	0	0	0	24	0	0	0	0	0	0	0	73	0	**376**	**97.7**
Pigs	Yes	0	0	0	1	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**2.3**
Pigs	**Total**	**0**	**0**	**0**	**63**	**0**	**0**	**9**	**216**	**0**	**0**	**0**	**24**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**73**	**0**	**385**	**100.0**
Domestic fowl	No	0	0	0	155	0	0	0	370	0	0	0	0	0	0	0	0	0	0	0	0	0	**525**	**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**	**0.0**
Domestic fowl	**Total**	**0**	**0**	**0**	**155**	**0**	**0**	**0**	**370**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**525**	**100.0**
Other birds	No	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0	0	0	0	0	0	0	**36**	**100.0**
Other birds	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Other birds	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**36**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**36**	**100.0**
Xenopus	No	481	0	0	0	0	0	15	465	0	0	0	0	0	0	0	0	0	0	0	0	0	**961**	**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**	**0.0**
Xenopus	**Total**	**481**	**0**	**0**	**0**	**0**	**0**	**15**	**465**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**961**	**100.0**
Other amphibians	No	0	0	0	0	0	0	0	805	0	0	0	0	0	0	0	0	0	0	0	0	0	**805**	**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**	**0.0**
Other amphibians	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**805**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**805**	**100.0**
Zebra fish	No	14,524	4,395	0	0	554	734	3,628	42,670	0	1,124	391	2,055	0	1,397	0	1,309	0	1,551	0	2,794	14,864	**91,990**	**97.7**
Zebra fish	Yes	0	0	67	0	0	0	0	1,752	0	0	0	177	0	0	0	0	0	0	0	200	0	**2,196**	**2.3**
Zebra fish	**Total**	**14,524**	**4,395**	**67**	**0**	**554**	**734**	**3,628**	**44,422**	**0**	**1,124**	**391**	**2,232**	**0**	**1,397**	**0**	**1,309**	**0**	**1,551**	**0**	**2,994**	**14,864**	**94,186**	**100.0**
Salmon, trout, chars and graylings	No	0	0	0	0	0	0	1,254	0	0	0	0	0	0	0	0	500	0	0	0	0	0	**1,754**	**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**	**0.0**
Salmon, trout, chars and graylings	**Total**	**0**	**0**	**0**	**0**	**0**	**0**	**1,254**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**500**	**0**	**0**	**0**	**0**	**0**	**1,754**	**100.0**
Other fish	No	214	0	0	0	0	0	0	2,456	0	0	0	0	0	0	0	0	0	0	0	578	0	**3,248**	**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**	**0.0**
Other fish	**Total**	**214**	**0**	**0**	**0**	**0**	**0**	**0**	**2,456**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**578**	**0**	**3,248**	**100.0**
All Species	**No**	**28,259**	**28,583**	**0**	**8,403**	**8,057**	**12,082**	**32,333**	**177,331**	**511**	**1,301**	**451**	**5,832**	**289**	**1,397**	**3,808**	**3,698**	**421**	**2,025**	**90**	**22,095**	**19,740**	**356,706**	**99.0**
All Species	**Yes**	**0**	**5**	**67**	**1**	**21**	**0**	**659**	**2,037**	**0**	**0**	**0**	**200**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**782**	**0**	**3,772**	**1.0**
All Species	**Total**	**28,259**	**28,588**	**67**	**8,404**	**8,078**	**12,082**	**32,992**	**179,368**	**511**	**1,301**	**451**	**6,032**	**289**	**1,397**	**3,808**	**3,698**	**421**	**2,025**	**90**	**22,877**	**19,740**	**360,478**	**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	Luxembourg	Netherlands	Norway	Portugal	Romania	Slovakia	Spain	Sweden	Total	%
Mice	No	5,566	13,962	1,203	1	35	61,050	130,890	1,803	1,115	183	11,053	8,023	2,546	131	215	34,437	1,225	**273,438**	**97.7**
Mice	Yes	0	79	0	0	0	0	6,394	0	0	0	0	0	0	0	0	0	0	**6,473**	**2.3**
Mice	**Total**	**5,566**	**14,041**	**1,203**	**1**	**35**	**61,050**	**137,284**	**1,803**	**1,115**	**183**	**11,053**	**8,023**	**2,546**	**131**	**215**	**34,437**	**1,225**	**279,911**	**100.0**
Rats	No	0	719	0	0	0	7,995	1,110	0	0	0	274	6	0	0	89	0	0	**10,193**	**100.0**
Rats	Yes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
Rats	**Total**	**0**	**719**	**0**	**0**	**0**	**7,995**	**1,110**	**0**	**0**	**0**	**274**	**6**	**0**	**0**	**89**	**0**	**0**	**10,193**	**100.0**
Dogs	No	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	**7**	**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**	**7**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7**	**100.0**
Zebra fish	No	0	0	0	0	0	509	16,853	216	0	0	119	6,351	0	0	0	0	0	**24,048**	**95.6**
Zebra fish	Yes	0	0	0	0	0	0	1,096	0	0	0	0	0	0	0	0	0	0	**1,096**	**4.4**
Zebra fish	**Total**	**0**	**0**	**0**	**0**	**0**	**509**	**17,949**	**216**	**0**	**0**	**119**	**6,351**	**0**	**0**	**0**	**0**	**0**	**25,144**	**100.0**
Other fish	No	0	0	0	0	0	0	573	0	0	0	0	0	0	0	0	0	0	**573**	**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**	**0**	**573**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**573**	**100.0**
All Species	**No**	**5,566**	**14,681**	**1,203**	**1**	**35**	**69,561**	**149,426**	**2,019**	**1,115**	**183**	**11,446**	**14,380**	**2,546**	**131**	**304**	**34,437**	**1,225**	**308,259**	**97.6**
All Species	**Yes**	**0**	**79**	**0**	**0**	**0**	**0**	**7,490**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**7,569**	**2.4**
All Species	**Total**	**5,566**	**14,760**	**1,203**	**1**	**35**	**69,561**	**156,916**	**2,019**	**1,115**	**183**	**11,446**	**14,380**	**2,546**	**131**	**304**	**34,437**	**1,225**	**315,828**	**100.0**