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COMMISSION STAFF WORKING DOCUMENT

Summary report on statistical information on the use of animals for scientific purposes in the Member States of the European Union and Norway in 2023

Summary report on statistical information on the use of animals in procedures

Summary report on statistical information on the use of animals in procedures

This year's report has been streamlined and simplified, as all the underlying data are now publicly accessible through the ALURES (**A**nima**L** **U**se **R**eporting – **E**U **S**ystem) Statistical EU Database[1], giving users direct access to the detailed data. If any of the numbers cited are not set out in the tables or figures in this report, you can access them directly in the ALURES database using filters[2].

I. Abstract

The 2023 report continues to track the use of animals for scientific purposes in the Union, including for basic and applied research, regulatory testing, routine production, education and training. Meaningful progress has been made under the Directive, with clear downward trends in animal use (Figure 1), especially for regulatory and basic research purposes.

The total number of animals used for the first time dropped again in 2023 to around 7.97 million (down by 4.9% since 2022 and by 9.6% since 2018)[3], putting it at the same level as in 2020 during the COVID-19 pandemic. First use of non-human primates fell sharply (-25%) compared to 2022 – a welcome development – although it is too soon to consider this decrease a consistent long-term trend.

In 2023, the total number of all animal uses for research, testing, routine production and education decreased to 8.08 million (down by 4.7% since 2022 and by 10.1% since 2018) (Figure 1). Uses for regulatory testing have continued to drop but at a slower pace. In 2023, although regulatory uses did not decrease greatly compared to 2022 (-4.6%), there has been a significant decrease since 2018 (-35.1%). In fact, compared to 2018, there was a clear decrease in uses for legislation on medicinal products for human use (-52%), food legislation including food contact materials (-81.3%) and other legislation (-53.2%).

However, the use of animals for testing industrial chemicals has not fallen; it is gradually increasing. Despite this, there has been a significant decrease in animal use for several regulatory testing areas where alternatives were adopted over the last five years. These include batch potency testing (-39.6%), batch safety testing (-38.1%), safety testing in the food and feed sector (-85.1%), pyrogenicity testing (-40.4%) and skin irritation/corrosion testing (-29.6%) (Table 11).

In 2023, the number of severe procedures continued to fall, accounting for less than 9% of all uses, down from nearly 11% in 2018. This report includes a dedicated analysis of severity levels, which is essential for a clear understanding of their impact on animals.

[1] ALURES Statistical EU database.

[2] This is why the 'EU data tables' located in Part B of reports for previous years are no longer provided but can be consulted in the ALURES database.

[3] 2018 was chosen as the reference year because it was the first year to include data from Norway and the fourth year for other Member States under Directive 2010/63/EU, thus providing more robust data for making a comparison. The numbers initially reported by the United Kingdom have been excluded from this report.

Although animal reuse rose somewhat in 2023 (by 10.7% compared to 2022), it remained well below the 2018 level (-34.7%).

The routine production of monoclonal antibodies using the mouse ascites method, resulting in severe uses, decreased by -27% compared to 2022 but remained high overall. Mouse ascites method is almost entirely carried out in France.

There continued to be significant fluctuations in the numbers reported for the maintenance of colonies of genetically altered animals, largely due to changes in how individual countries – particularly France – were collecting data. Since 2022, a great effort has been made to improve data accuracy.

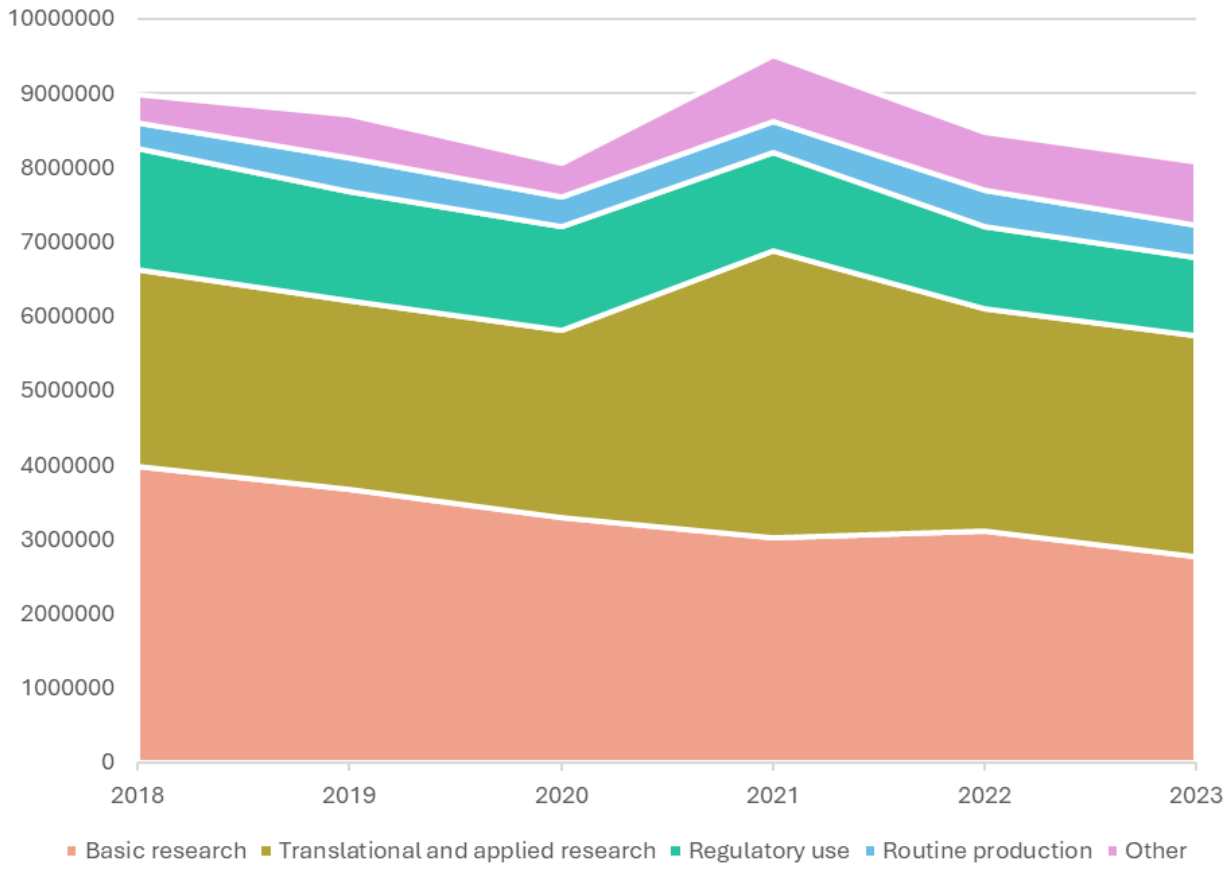

Figure 1: Number of animal uses by main scientific purpose between 2018 and 2023

II. Compilation and overview of EU data between 2018 and 2023

This report aims to provide a comprehensive overview of the use of animals in procedures in the European Union and Norway in 2023. It provides data in the figures or in summary tables illustrating a specific aspect of the Directive. Overall numbers are given for 2023. A systematic trend analysis also provides information on the progress made towards the Directive's objectives by comparing 2023 data with 2022 data to show short-term changes, and with 2018 data to show longer-term changes.

The European Commission has significantly improved transparency and accessibility through its open-access ALURES database. As of May 2025, anyone interested can now download comprehensive data sets for in-depth analysis using various data-mining tools. This streamlines the reporting process by eliminating unnecessary repetition where there have been no significant changes, so that the focus is more on key figures and major developments.

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

This section focuses on animal numbers, i.e. how many animals are used *for the first time* in procedures for the purposes of research, testing, routine production and education. It excludes the reuse of animals, which is examined in more detail in Section II.2.3, looking at all uses of animals by purpose and other use-related information. Section II.3 covers the use of animals for the creation of new genetically altered lines or for the maintenance of colonies of established genetically altered animal lines. However, animals used for research, testing, routine production and educational purposes may be conventional or genetically altered.

In addition to providing numbers on the animals used, this section also provides information on each species in relation to their origin. For non-human primates, it gives information on the progress made towards higher-generation purpose-bred animals, by recording the generation and the type of colony from which a non-human primate is sourced.

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

In 2023, the total number of animals used for the first time in the Union was 7.97 million, down by 4.9% since 2022 and by 9.6% compared to 2018, confirming a slow but steady decrease (Table 1).

This was mainly due to a decrease in mammal use compared to 2018, especially in the use of mice (-19.3%) and rats (-30.5%). The decreasing trend was contrasted with an increase in the use of fish (+11.9%), in particular salmon, trout, chars, graylings and sea bass. There was a significant increase in cephalopods used for the first time in 2023 (+163.6% compared to 2022). However, although there were significant proportional increases for both reptiles and cephalopods, the actual numbers of animals involved remained small (Table 2).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Mammals	5,885,013 (66.7%)	5,289,886 (63.1%)	**4,743,775 (59.5%)**	-10.3%	-19.4%
Birds	436,316 (4.9%)	517,011 (6.2%)	**426,629 (5.4%)**	-17.5%	-2.2%
Reptiles	1,544 (0%)	4,631 (0.1%)	**3,339 (0%)**	-27.9%	+116.3%
Amphibians	24,412 (0.3%)	32,924 (0.4%)	**27,925 (0.4%)**	-15.2%	+14.4%
Fish	2,470,851 (28%)	2,538,269 (30.3%)	**2,765,503 (34.7%)**	+9%	+11.9%
Cephalopods	4,268 (0%)	2,676 (0%)	**7,055 (0.1%)**	+163.6%	+65.3%
Total	**8,822,404 (100%)**	**8,385,397 (100%)**	**7,974,226 (100%)**	**-4.9%**	**-9.6%**

Table 1: Numbers of animals used for the first time by main types of species in 2018, 2022 and 2023

Out of this total, 59.5% of all animals used for the first time were mammals (a decrease of 7.2 percentage points compared to 2018), 34.7% were fish (an increase of 6.7 percentage points compared to 2018) and 5.4% were birds.

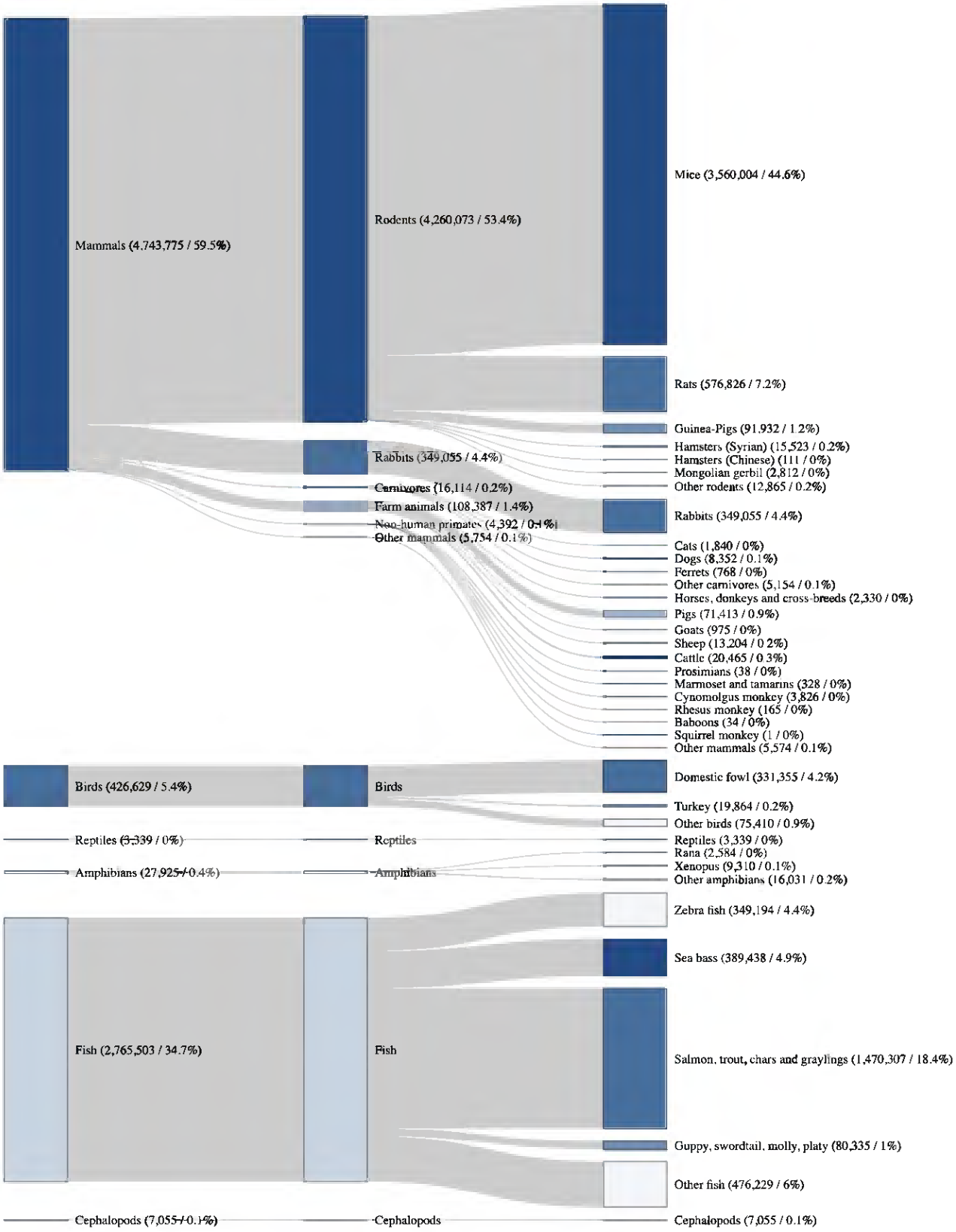

Figure 2: Number of animals used for the first time by main classes of species in 2023

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Mammals					
Rodents					
Mice	4,410,737	4,010,766	**3,560,004**	-11.2%	-19.3%
Rats	829,906	625,777	**576,826**	-7.8%	-30.5%
Guinea Pigs	123,486	85,167	**91,932**	+7.9%	-25.6%
Hamsters (Syrian)	9,397	21,613	**15,523**	-28.2%	+65.2%
Hamsters (Chinese)	20	31	**111**	+258.1%	+455%
Mongolian gerbil	4,269	3,440	**2,812**	-18.3%	-34.1%
Other rodents	19,534	15,260	**12,865**	-15.7%	-34.1%
Rabbits					
Rabbits	332,097	372,239	**349,055**	-6.2%	+5.1%
Carnivores					
Cats	1,517	1,409	**1,840**	+30.6%	+21.3%
Dogs	14,802	8,709	**8,352**	-4.1%	-43.6%
Ferrets	1,041	899	**768**	-14.6%	-26.2%
Other carnivores	4,267	840	**5,154**	+513.6%	+20.8%
Farm animals					
Horses, donkeys and cross-breeds	1,626	4,110	**2,330**	-43.3%	+43.3%
Pigs	79,699	86,953	**71,413**	-17.9%	-10.4%
Goats	1,443	1,268	**975**	-23.1%	-32.4%
Sheep	17,398	15,909	**13,204**	-17%	-24.1%
Cattle	22,580	21,434	**20,465**	-4.5%	-9.4%
Non-human primates					
Prosimians	170	74	**38**	-48.6%	-77.6%
Marmoset and tamarins	289	495	**328**	-33.7%	+13.5%
Cynomolgus monkey	5,349	4,950	**3,826**	-22.7%	-28.5%
Rhesus monkey	210	140	**165**	+17.9%	-21.4%
Vervets (Chlorocebus spp.)	16	48	**0**	-100%	-100%
Baboons	30	64	**34**	-46.9%	+13.3%
Squirrel monkey	25	13	**1**	-92.3%	-96%
Other species of Old World Monkeys (Cercopithecoidea)	22	0	**0**	NA	-100%
Other mammals					
Other mammals	5,083	8,278	**5,754**	-30.5%	+13.2%
Birds					
Domestic fowl	341,763	417,903	**331,355**	-20.7%	-3%
Turkey	0	18,577	**19,864**	+6.9%	NA
Other birds	94,553	80,531	**75,410**	-6.4%	-20.2%
Reptiles					
Reptiles	1,544	4,631	**3,339**	-27.9%	+116.3%
Amphibians					
Rana	3,563	2,638	**2,584**	-2%	-27.5%
Xenopus	14,074	15,720	**9,310**	-40.8%	-33.8%
Other amphibians	6,775	14,566	**16,031**	+10.1%	+136.6%
Fish					
Zebra fish	259,468	362,449	**349,194**	-3.7%	+34.6%
Sea bass	0	116,706	**389,438**	+233.7%	NA
Salmon, trout, chars and graylings	0	1,289,139	**1,470,307**	+14.1%	NA
Guppy, swordtail, molly, platy	0	78,388	**80,335**	+2.5%	NA
Other fish	2,211,383	691,587	**476,229**	-31.1%	-78.5%
Cephalopods					
Cephalopods	4,268	2,676	**7,055**	+163.6%	+65.3%
Totals					
Total	**8,822,404**	**8,385,397**	**7,974,226**	**-4.9%**	**-9.6%**

Table 2: Numbers of animals used for the first time by types of species in 2018, 2022 and 2023

Figure 2 shows the breakdown of species used for the first time in 2023 by types of species. Mice are the most used species (44.6%), followed by salmon, trout, chars and graylings (18.4%), rats (7.2%), sea bass (4.8%), zebra fish (4.4%), rabbits (4.4%) and domestic fowl (4.1%).

In 2023, there was a significant decrease in first uses of non-human primates (down by 24.1%) compared to 2022; despite two consecutive increases in 2021 and 2022 (Table 3), there was a similar decrease when compared to 2018 (-28.1%). The only increase compared to 2022 in the use of species where over 50 animals were used for the first time was for rhesus monkeys (+17.9%). During this same period, the first uses of all other species of non-human primates decreased, notably of marmoset and tamarins (-33.7%) and cynomolgus monkeys (-22.7%), the two most used species (Table 2).

The use of four out of six other types of species decreased compared to 2022: 'other fish' (-31.1%), 'other mammals' (-30.5%), 'other rodents' (-15.7%) and 'other birds' (-6.3). The remaining two categories increased: 'other carnivores' (by 513.5%, note that France changed the classification of bats from 'other mammals' to 'other carnivores', triggering a decrease of the former and increase of the latter), and 'other amphibians' (+10.0%). Some examples of the main species classified as 'other' species are *ambystoma mexicanum* ('other amphibians'), *parus major* ('other birds'), bats ('other carnivores'), tuna fish ('other fish'), *capreolus capreolus* ('other mammals') and *clethrionomys glareolus* ('other rodents') (Table 2).

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

The origin (place of birth) of animals is divided into two categories: species in the category of non-human primates and other species. More detailed information is collected on the origin of non-human primates (continent of origin), their generation and the type of colony from which the animal is sourced (see II.1.2.2.2).

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

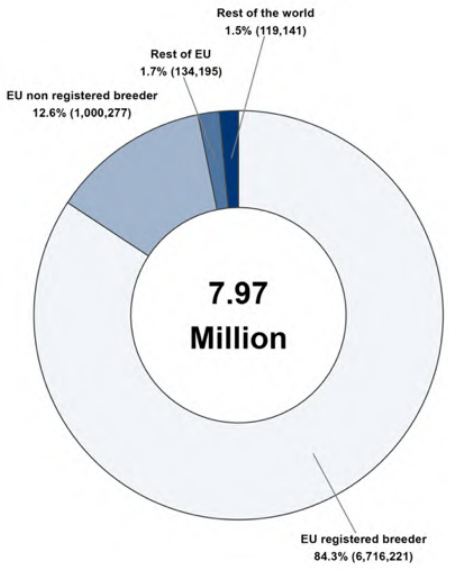

Figure 3: Place of birth of animals other than non-human primates in 2023

In 2023, 84.3% of the animals used for scientific purposes for the first time were born in the EU at registered breeders[4], 12.6% were born in the EU but at non-registered breeders and 3.2% were born outside of the EU (either in the rest of Europe or outside of Europe) (Figure 3).

Annex I to the Directive contains a list of animals that may only be used if the animals have been bred for use in procedures (see Article 10). The data for Annex I and non-Annex I species have been broken down for the first time in this report.

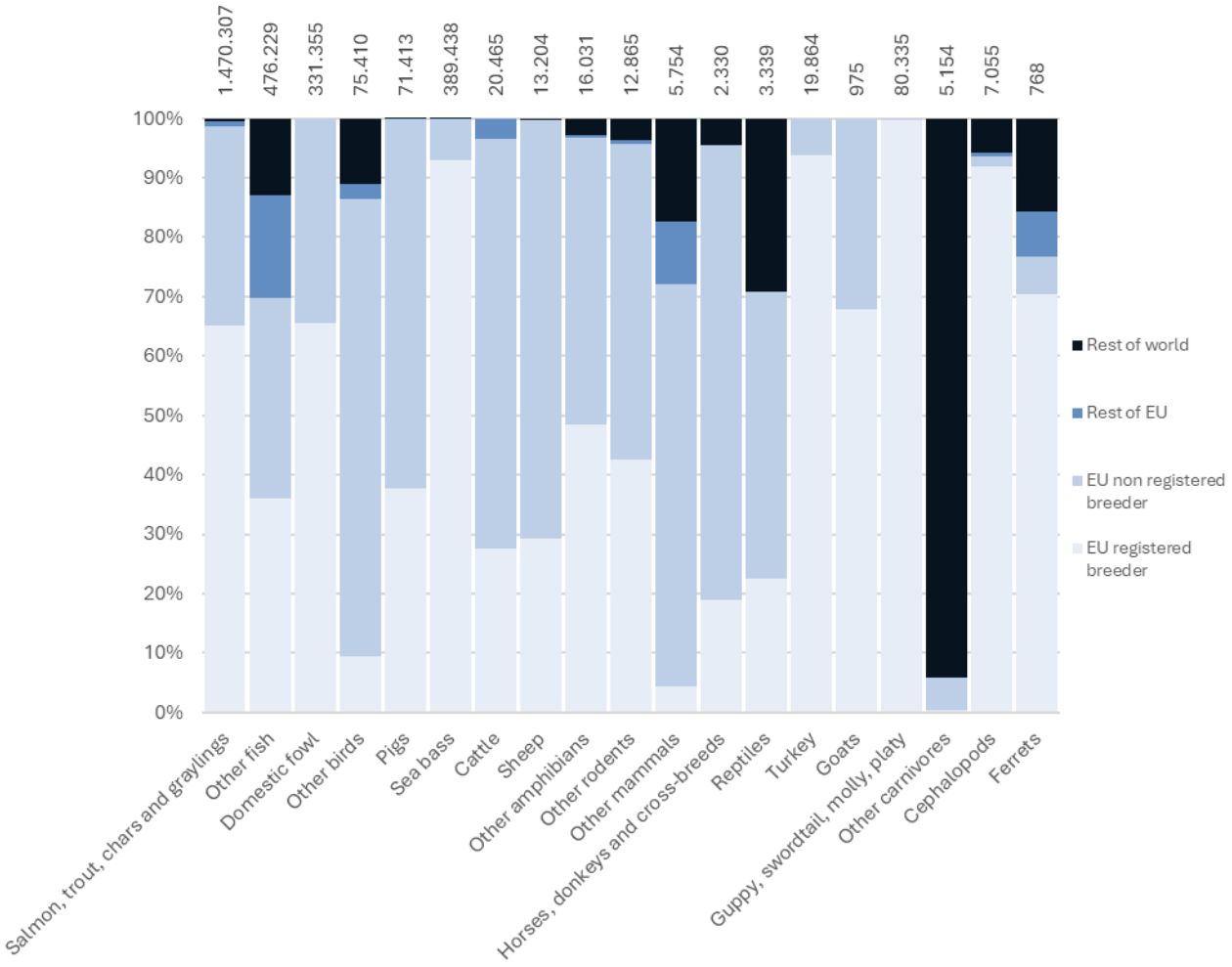

Figure 4: Proportion of animals not listed in Annex I used for the first time by origin

Most animals born at non-EU-registered breeders were non-Annex I species (Figure 4). These were mainly farm animals and fish, together representing more than 85% of all animals reported as sourced from non-EU-registered breeders. The largest proportion were fish, including salmonids (salmon, trout, chars and graylings; ≈ 0.5 million; 52%) and other fish (≈ 0.16 million; 17%). These types of species

[4] This includes animals born at registered breeders in Norway authorised under Article 20 of Directive 2010/63/EU.

may include both farmed and wild-caught fish. Domestic fowl accounted for approximately 12%, followed by other birds (6%), pigs (5%), cattle (1.5%) and sheep (1%). Farm animals are generally not purpose-bred for research purposes but are sourced from agricultural holdings.

Annex I species must be purpose-bred unless an exemption for scientific reasons was authorised. Breeders of Annex I species (if breeding for scientific purposes) must be registered in the EU. If sourced from the EU but from a non-registered breeder, the animal has not been purpose-bred. However, when sourced from the rest of Europe or outside Europe, they may be either purpose-bred or not. It is therefore helpful to examine Annex I species born at non-registered breeders in the EU.

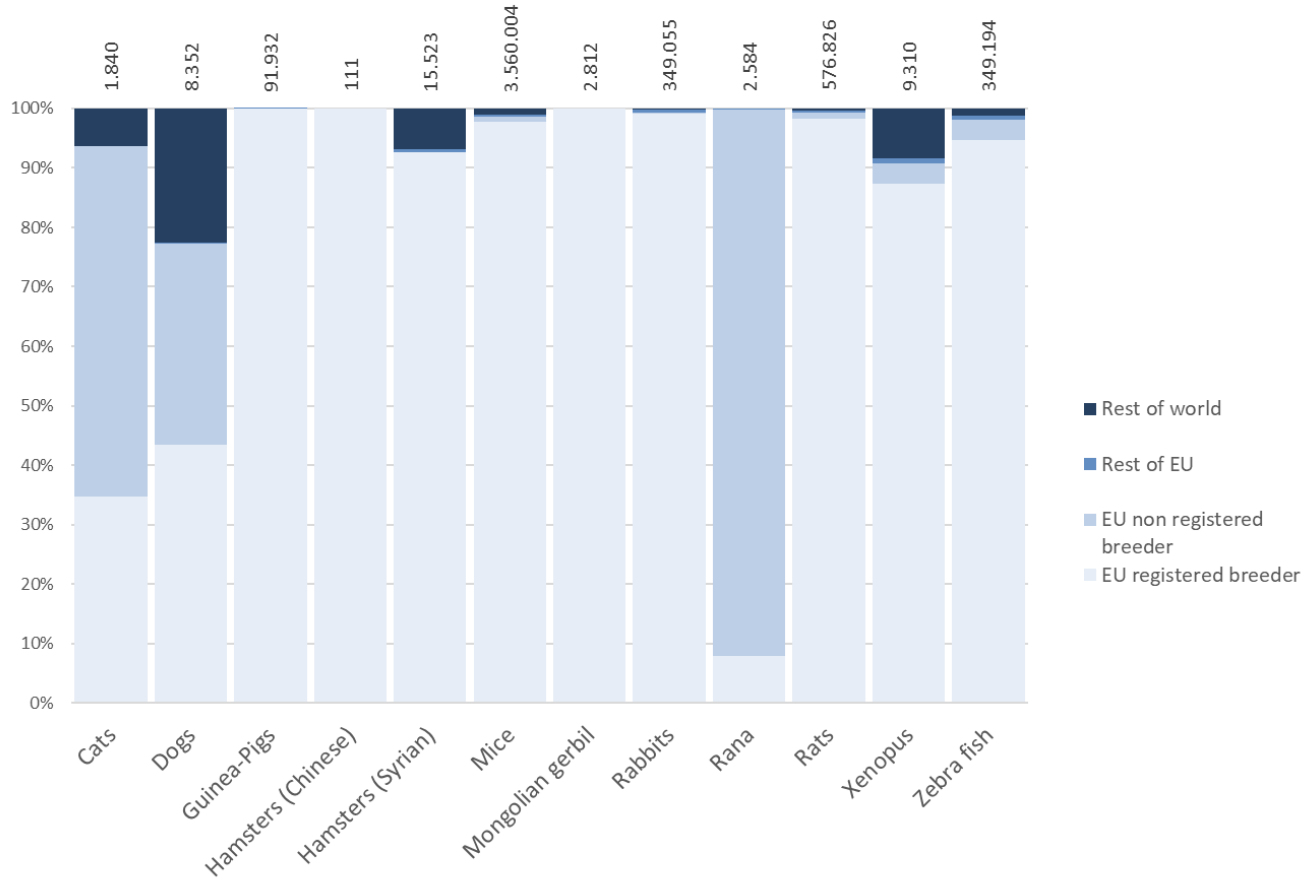

Figure 5: Proportion of animals listed in Annex I used for the first time by origin

Most Annex I animals (98%) were born at a registered breeder in the EU with the exception of rana, cats and dogs (Figure 5). Rana sourced from non-registered breeders in the EU were reported only by Germany (56%), Belgium (34%) and Finland (10%). 86% of cats sourced from non-registered breeders in the EU was reported by Latvia (31%), Finland (21%), Belgium (20%) and Sweden (14%). 84% of dogs in this category were reported by four countries: Germany (25%), Finland (24%), Spain (21%) and Sweden (14%). This category included pet dogs and cats participating in patient studies involving minimal interventions such as a single blood sample, as reported by Finland.

II.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 a high risk of injury and suffering during capture and transport. To end the capture of animals from the wild, including for the purposes of breeding, the Directive aims to shift practices towards using the offspring of non-human primates which have been bred in captivity or where they are sourced from self-sustaining colonies, i.e. from parents that have been bred in captivity (see Article 10 of the Directive).

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

II.1.2.2.1. Non-human primates - source

In 2023, non-human primates came from registered breeders in the EU, Asia and Africa. No non-human primates were sourced from America or elsewhere in 2023.

	2018	2019	2020	2021	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Animals born at a registered breeder within EU	842 (13.8%)	684 (12.9%)	643 (13.4%)	669 (12.6%)	745 (12.9%)	612 (13.9%)	-17.9%	-27.3%
Animals born in rest of Europe	0 (0%)	0 (0%)	1 (0%)	0 (0%)	120 (2.1%)	0 (0%)	-100%	NA
Animals born in Asia	2,737 (44.8%)	2,418 (45.5%)	1,584 (33.1%)	2,034 (38.3%)	1,942 (33.6%)	1,798 (40.9%)	-7.4%	-34.3%
Animals born in America	12 (0.2%)	18 (0.3%)	36 (0.8%)	24 (0.5%)	46 (0.8%)	0 (0%)	-100%	-100%
Animals born in Africa	2,466 (40.4%)	1,917 (36%)	2,291 (47.9%)	2,587 (48.7%)	2,931 (50.7%)	1,982 (45.1%)	-32.4%	-19.6%
Animals born elsewhere	54 (0.9%)	282 (5.3%)	229 (4.8%)	0 (0%)	0 (0%)	0 (0%)	NA	-100%
Total	**6,111 (100%)**	**5,319 (100%)**	**4,784 (100%)**	**5,314 (100%)**	**5,784 (100%)**	**4,392 (100%)**	**-24.1%**	**-28.1%**

Table 3: Changes in non-human primate sources between 2018 and 2023

Since 2018, the proportion of non-human primates born at an EU-registered breeder has remained stable, at 13.9% in 2023. The number of non-human primates coming from Africa in 2023 decreased by 32.4% to about 1 000 animals. This is a significant change, as numbers had increased in 2021 and 2022. However, no clear trend has emerged since 2018 regarding the origin of non-human primates (Table 3).

In 2023, the largest proportion of non-human primates used for the first time were still cynomolgus monkeys (87.1%), sourced almost entirely from outside the EU (Table 4). By contrast, other species of non-human primates were almost exclusively sourced from EU-registered breeders (all but five rhesus monkeys).

	Animals born at a registered breeder within EU	Animals born in Asia	Animals born in Africa
Prosimians	38 (100%)	0 (0%)	0 (0%)
Marmoset and tamarins	328 (100%)	0 (0%)	0 (0%)
Cynomolgus monkey	51 (1.3%)	1,793 (46.9%)	1,982 (51.8%)
Rhesus monkey	160 (97%)	5 (3%)	0 (0%)
Baboons	34 (100%)	0 (0%)	0 (0%)
Squirrel monkey	1 (100%)	0 (0%)	0 (0%)
Total	**612 (13.9%)**	**1,798 (40.9%)**	**1,982 (45.1%)**

Table 4: Source of non-human primates by species in 2023

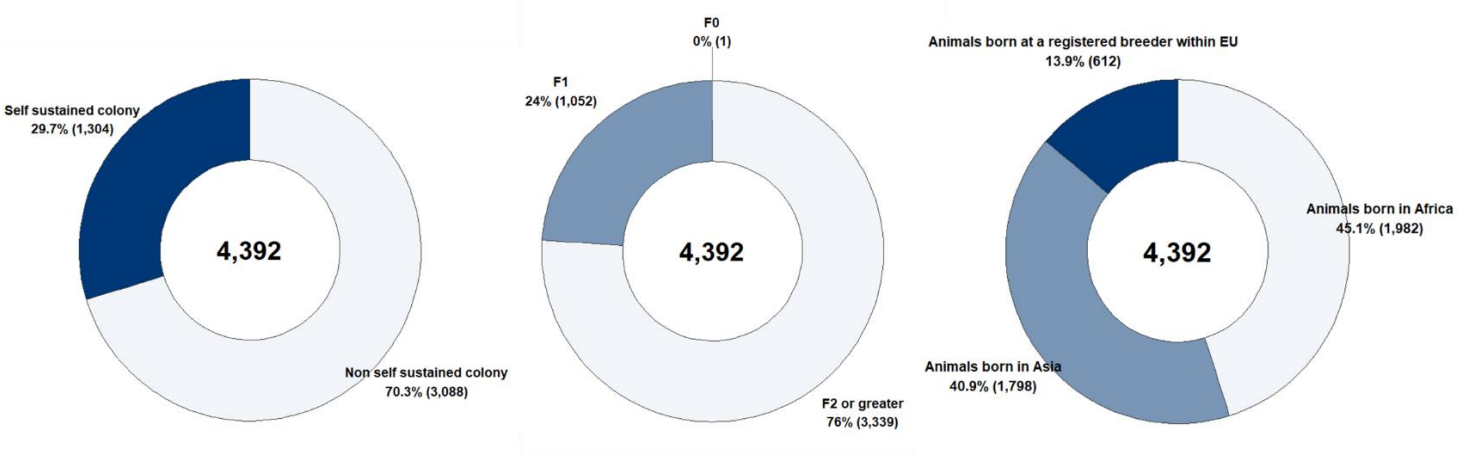

Figure 6: Origin and source of non-human primates used for the first time in 2023 by (left) source of colony (self-sustained vs non-self-sustained), (centre) generation and (right) place of birth

II.1.2.2.2. Non-human primates – colonies and generation

From 2021 onwards, the reporting on whether a non-human primate was sourced from a self-sustaining colony was separated from the reporting on its generation (F0, F1, F2 or greater). In 2023, most of the non-human primates bred in captivity came from self-sustained colonies (70.3%), a slight decrease compared to 2022 (down by 4.4 percentage points) (Figure 6; Table 5).

	Self-sustained colony	Non-self-sustained colony
Prosimians	37 (97.4%)	1 (2.6%)
Marmoset and tamarins	269 (82%)	59 (18%)
Cynomolgus monkey	2,585 (67.6%)	1,241 (32.4%)
Rhesus monkey	162 (98.2%)	3 (1.8%)
Baboons	34 (100%)	0 (0%)
Squirrel monkey	1 (100%)	0 (0%)
Total	**3,088 (70.3%)**	**1,304 (29.7%)**

Table 5: Non-human primates colony type by species in 2023

In 2023, more than half of the non-human primates born in Africa originated from self-sustained colonies (54.5%), although a substantial proportion (45.5%) still came from non-self-sustained colonies. This is in stark contrast to the animals born in Asia and within the EU, where most were sourced from self-sustained colonies (81.6% and 88.2% respectively) (Table 6).

	Self-sustained colony	Non-self-sustained colony
Animals born at a registered breeder within EU	540 (88.2%)	72 (11.8%)
Animals born in Asia	1,468 (81.6%)	330 (18.4%)
Animals born in Africa	1,080 (54.5%)	902 (45.5%)
Total	**3,088 (70.3%)**	**1,304 (29.7%)**

Table 6: Non-human primates colony type by source in 2023

Most non-human primates are purpose-bred second generation or higher (76%). Of the non-human primates born at a registered breeder in the EU or in Asia, less than 1.7% of non-human primates used for the first time were first generation. In Africa, first-generation animals represented 51.4% of the non-human primates used in 2023 (Table 7). One non-human primate was reported as being wild-caught (F0). This animal, reported by France, was caught in 1996 and was part of a long-term project to investigate prion transmission risks in transfusions. For the first time in 2023, no non-human primates were reported as being born in America.

	F2 or greater	F1	F0
Animals born at a registered breeder within EU	609 (99.5%)	3 (0.5%)	0 (0%)
Animals born in Asia	1,767 (98.3%)	31 (1.7%)	0 (0%)
Animals born in Africa	963 (48.6%)	1,018 (51.4%)	1 (0.1%)
Total	**3,339 (76%)**	**1,052 (24%)**	**1 (0%)**

Table 7: Generation of non-human primates by source in 2023

II.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 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 on the severity experienced by the animals, their genetic status and reuse. Information on the use of animals to satisfy regulatory requirements is also collected.

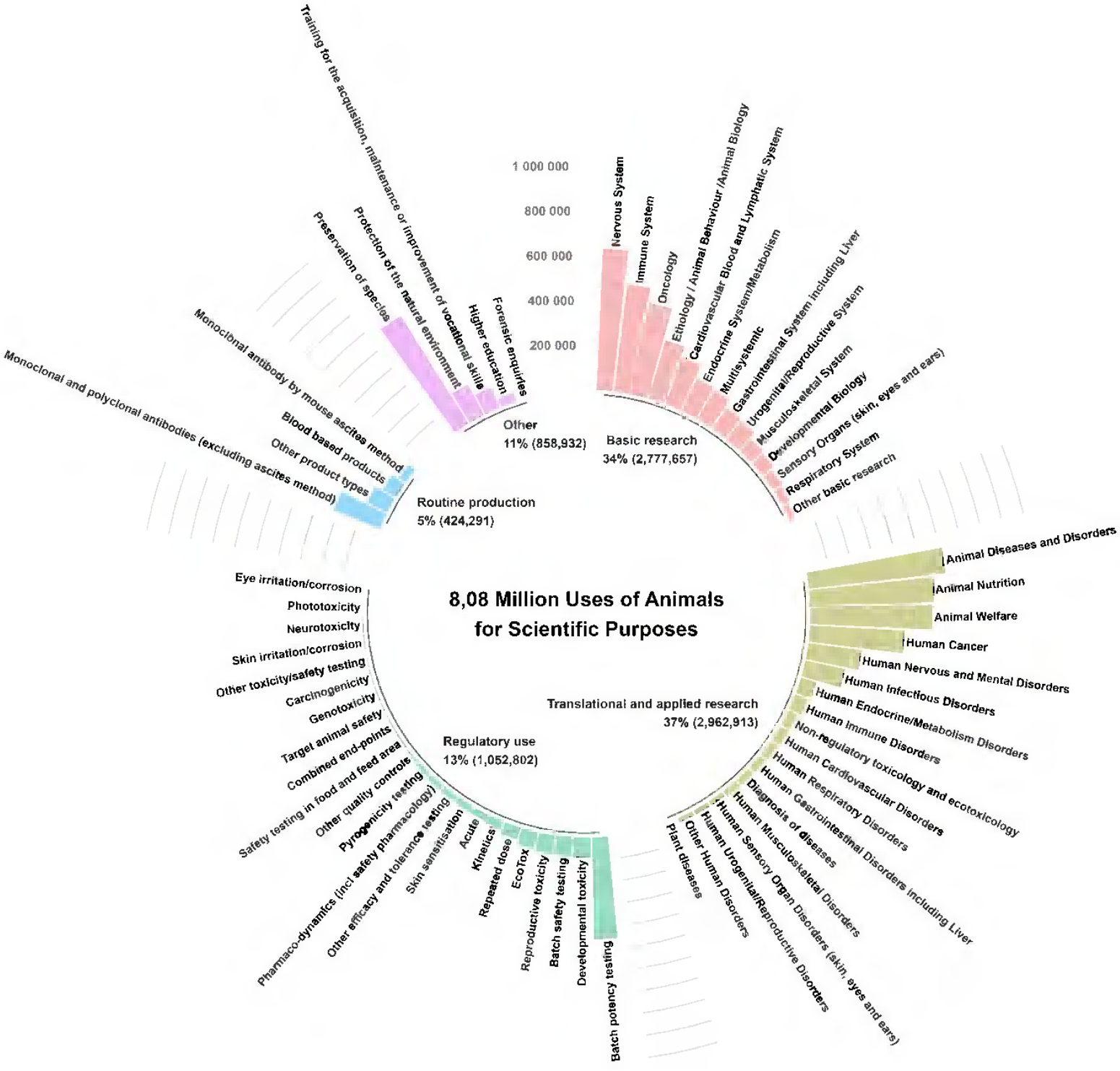

Figure 7: All uses of animals for research and testing in 2023

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

In 2023, a total 8.08 million animal uses were recorded (including first use and any subsequent reuse) for the purposes of research, testing, routine production and education (Figure 7). This is a drop of 4.7% compared to 2022 and 10.1% lower than in 2018 (Table 8).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Basic research	3,982,963 (44.4%)	3,113,850 (36.7%)	**2,777,657 (34.4%)**	-10.8%	-30.3%
Translational and applied research	2,650,730 (29.5%)	2,993,795 (35.3%)	**2,962,913 (36.7%)**	-1%	+11.8%
Regulatory use	1,622,816 (18.1%)	1,103,612 (13%)	**1,052,802 (13%)**	-4.6%	-35.1%
Routine production	354,209 (3.9%)	477,632 (5.6%)	**424,291 (5.3%)**	-11.2%	+19.8%
Other	368,363 (4.1%)	788,956 (9.3%)	**858,932 (10.6%)**	+8.9%	+133.2%
Total	**8,979,081 (100%)**	**8,477,845 (100%)**	**8,076,595 (100%)**	**-4.7%**	**-10.1%**

Table 8: Total number of uses of animals by main purpose categories in 2018, 2022 and 2023

II.2.1.1. Main categories of use for scientific purposes

In 2023, most uses of animals were conducted for research purposes (71.1%). Of this, 34.4% of uses were carried out for basic research and 36.7% for translational and applied research purposes. A further 13% of animal uses in procedures were carried out to meet regulatory requirements, while only 5.3% of uses was for routine production. Other categories (10.6%) included the protection of the natural environment in the interest of human or animal health or welfare, preservation of species, higher education, training for the acquisition, maintenance or improvement of vocational skills, and forensic enquiries (Table 8).

Looking at trends over time, uses for basic research showed a significant drop for the first time between 2023 and 2022 (-10.8%). Although it may be subject to fluctuations from one year to the next, this drop amounted to -30.3% when compared with 2018. This trend should be monitored over the next few years. The trend for applied and translational research, however, has remained stable (-1% compared to 2022), making it the most important sector for animal uses for scientific purposes. Regulatory uses have continued to decrease over time (down by 4.6% since 2022), confirming the general trend (down by 35.1% since 2018). Routine production also decreased compared to 2022 (by 11.2%) but showed an overall increase of +19.8% compared to 2018. Other categories increased by 8.9%, compared to 2022, mainly due to an increase in procedures carried out in Norway on various fish species for the preservation of species.

II.2.1.2. Severity of uses

The Directive requires reporting on the level of severity experienced by each animal when used for scientific purposes. In 2023, 51.9% of uses were reported as ‘mild’ (up to and including), 36.3% ‘moderate’ and 8.7% as ‘severe’, while 3.1% of uses were reported as ‘non-recovery’ (Table 9). Looking at the proportion of most severe uses, these have continued to fall, slowly but consistently (by 0.5 percentage points since 2022 and by 2.2 percentage points since 2018).

	2018	2022	2023
Non-recovery	521,765 (5.8%)	304,582 (3.6%)	**250,771 (3.1%)**
Mild [up to and including]	4,311,312 (48%)	3,789,476 (44.7%)	**4,192,231 (51.9%)**
Moderate	3,169,559 (35.3%)	3,599,739 (42.5%)	**2,930,132 (36.3%)**
Severe	976,445 (10.9%)	784,048 (9.2%)	**703,461 (8.7%)**
Total	**8,979,081 (100%)**	**8,477,845 (100%)**	**8,076,595 (100%)**

Table 9: Severity of uses reported in 2023

Since the level of severity experienced is linked to the type of use, and use patterns vary between Member States, it is not advisable to compare severities between Member States. For example, a Member State with a high proportion of animal uses for the purposes of regulatory testing is likely to have a higher proportion of severe uses than a Member State where the main uses are for education and training.

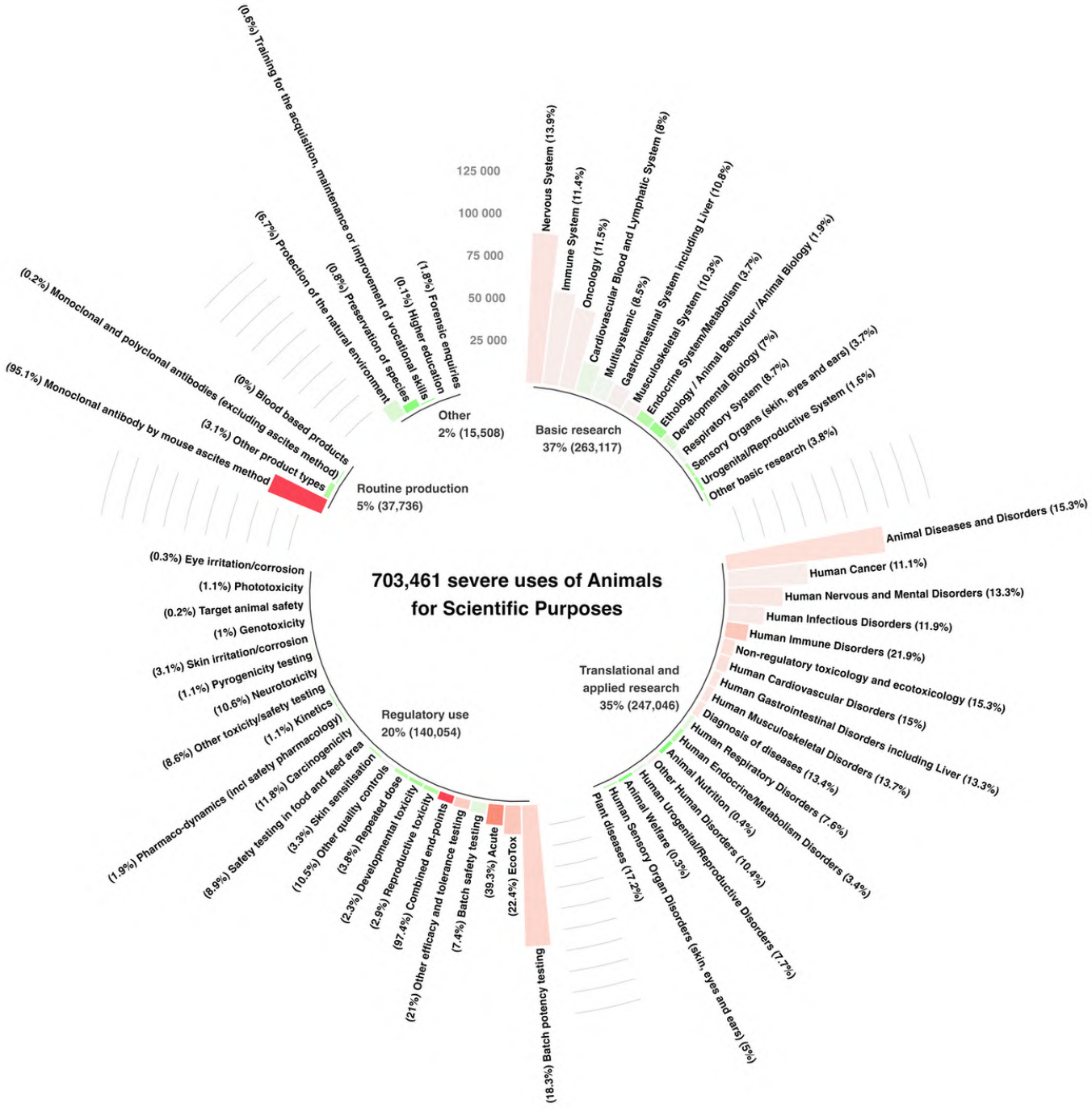

Figure 8: Severe uses of animals for research and testing in 2023

Figure 8 shows the number of severe uses in 2023 for each of the main purpose categories (size of bars). It also shows the number of severe uses as a proportion of the total number of uses (colour and percentages at the top of the bars).

An analysis of this figure indicates that, in absolute numbers, the highest number of severe uses were reported for research into animal diseases and disorders (93 931), the nervous system (88 292), batch potency testing (83 179), the immune system (55 077), oncology (47 507) and human cancer (46 897).

The highest proportion of severe uses were reported in: routine production of monoclonal antibodies by mouse ascites (95.1%); regulatory testing for combined end-points (97.4%), acute toxicity testing (39.3%), ecotoxicity testing (22.4%) and batch potency testing for quality checks (18.3%); basic research on the nervous system (13.9%); and translational and applied research on human immune disorders (21.9%).

The lowest number of severe uses were reported in: higher education (0.1%); training for the acquisition, maintenance or improvement of vocational skills (0.6%); preservation of species (0.8%); routine production of monoclonal and polyclonal antibodies, excluding the mouse ascites method (0.2%), and of blood-based products (less than 0.1%); regulatory use for target animal safety (0.2%) and eye irritation/corrosion (0.3%); and translational and applied research for animal welfare (0.3%).

For regulatory uses (Table 10), 13.3% were reported as severe, 22% moderate, 64.2% mild (up to and including) and 0.5% as non-recovery. Looking at specific types of legislation, although the total number of uses was not the most significant, the highest proportion of severe uses were in the area of feed legislation including legislation governing the safety of target animals, workers and the environment (56.7%), and biocides (37%).

	Non-recovery	Mild [up to and including]	Moderate	Severe
Legislation on medicinal products for human use	4,704 (0.9%)	310,366 (62.5%)	113,478 (22.9%)	67,894 (13.7%)
Legislation on medicinal products for veterinary use and their residues	34 (0%)	191,777 (69.1%)	44,861 (16.2%)	40,955 (14.8%)
Medical devices legislation	58 (0.1%)	14,093 (30.9%)	29,802 (65.4%)	1,612 (3.5%)
Industrial chemicals legislation	587 (0.3%)	117,991 (67.6%)	39,382 (22.6%)	16,674 (9.5%)
Plant protection product legislation	0 (0%)	14,479 (69.7%)	2,261 (10.9%)	4,036 (19.4%)
Biocides legislation	15 (1.2%)	430 (34.7%)	336 (27.1%)	458 (37%)
Food legislation including food contact material	0 (0%)	6,289 (80.7%)	802 (10.3%)	703 (9%)
Feed legislation including legislation for the safety of target animals, workers and environment	0 (0%)	2,351 (42%)	71 (1.3%)	3,173 (56.7%)
Other legislation	0 (0%)	18,023 (77.9%)	558 (2.4%)	4,549 (19.7%)
Total	**5,398 (0.5%)**	**675,799 (64.2%)**	**231,551 (22%)**	**140,054 (13.3%)**

Table 10: Severity of regulatory uses by type of legislation in 2023

Figure 9: Severe uses of animals by species and detailed purposes for research and testing in 2023

Looking at the detailed purposes for each category and type of animal species (Figure 9), we can highlight additional areas for concern where specific species are used with a high proportion of severe uses.

The main areas for concern, where animal species are used in high numbers (more than 5 000) and there is a high proportion of severe uses (above 20%), are regulatory testing and studies related to the immune system and related disorders. Specifically, this applies to:

- rodent uses for monoclonal antibody production by mice ascites (34 229 uses, 95%);
- fish uses for batch potency testing (16 822 uses, 48%);
- fish uses for studies on the immune system (15 989 uses, 32%);
- rodent uses for studies on human immune disorders (12 959 uses, 22%);
- rodent uses for LD50/LC50 testing (9 294 uses, 61%);
- fish uses for acute toxicity testing (7 087 uses, 25%).

Another main area for concern, where animal species are used in lower numbers (between 100 and 5 000) but where there is an even higher proportion of severe uses (above 30%), is regulatory testing. Specifically, this applies to:

- rodent uses for acute and sub-acute toxicity testing with other lethal methods (1 080 uses, 45%);
- rodent uses for combined end-point toxicity testing (4 667 uses, 97%);
- rabbit uses for other efficacy and tolerance testing (773 uses, 64%);
- carnivore uses for batch potency testing (123 uses, 38%);
- bird uses for other efficacy and tolerance testing (2 722 uses, 34%);
- amphibian uses for endocrine activity testing (320 uses, 31%);
- amphibian uses for the protection of species (1 600 uses, 62%);
- fish uses for non-regulatory toxicology and ecotoxicology (4 903 uses, 33%).

II.2.2. Detailed information on use purposes

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Basic research					
Oncology	432,191	495,488	**414,697**	-16.3%	-4%
Cardiovascular Blood and Lymphatic System	254,931	249,190	**220,360**	-11.6%	-13.6%
Nervous System	687,420	754,200	**632,968**	-16.1%	-7.9%
Respiratory System	52,932	40,030	**34,670**	-13.4%	-34.5%
Gastrointestinal System including Liver	141,132	145,692	**111,654**	-23.4%	-20.9%
Musculoskeletal System	76,753	70,123	**70,727**	+0.9%	-7.9%
Immune System	547,768	505,356	**482,026**	-4.6%	-12%
Urogenital/Reproductive System	83,859	75,838	**102,368**	+35%	+22.1%
Sensory Organs (skin, eyes and ears)	52,418	70,040	**45,918**	-34.4%	-12.4%
Endocrine System/Metabolism	187,421	159,984	**157,875**	-1.3%	-15.8%
Developmental Biology	0	56,178	**62,274**	+10.9%	NA
Multisystemic	309,585	179,636	**148,386**	-17.4%	-52.1%
Ethology / Animal Behaviour /Animal Biology	988,510	260,765	**270,037**	+3.6%	-72.7%
Other basic research	168,043	51,330	**23,697**	-53.8%	-85.9%
Translational and applied research					
Human Cancer	450,538	459,214	**423,387**	-7.8%	-6%
Human Infectious Disorders	190,731	191,861	**180,682**	-5.8%	-5.3%
Human Cardiovascular Disorders	66,479	50,945	**42,599**	-16.4%	-35.9%
Human Nervous and Mental Disorders	259,148	231,878	**239,236**	+3.2%	-7.7%
Human Respiratory Disorders	54,579	42,054	**36,830**	-12.4%	-32.5%
Human Gastrointestinal Disorders including Liver	37,868	32,039	**33,845**	+5.6%	-10.6%
Human Musculoskeletal Disorders	38,092	48,648	**30,691**	-36.9%	-19.4%
Human Immune Disorders	85,686	66,235	**59,235**	-10.6%	-30.9%
Human Urogenital/Reproductive Disorders	11,850	20,236	**20,965**	+3.6%	+76.9%
Human Sensory Organ Disorders (skin, eyes and ears)	35,175	24,934	**21,149**	-15.2%	-39.9%
Human Endocrine/Metabolism Disorders	115,102	84,469	**71,043**	-15.9%	-38.3%
Other Human Disorders	26,505	25,155	**20,479**	-18.6%	-22.7%
Animal Diseases and Disorders	937,444	400,918	**613,337**	+53%	-34.6%
Animal Nutrition	0	309,716	**550,713**	+77.8%	NA
Animal Welfare	137,426	915,652	**542,126**	-40.8%	+294.5%
Diagnosis of diseases	143,637	29,212	**31,038**	+6.3%	-78.4%
Plant diseases	38	10	**64**	+540%	+68.4%
Non-regulatory toxicology and ecotoxicology	60,432	60,619	**45,494**	-25%	-24.7%
Regulatory use					
Quality control (incl batch safety and potency testing)					
Batch safety testing	137,324	81,511	**85,035**	+4.3%	-38.1%
Pyrogenicity testing	29,815	19,168	**17,770**	-7.3%	-40.4%
Batch potency testing	752,958	452,699	**454,749**	+0.5%	-39.6%
Other quality controls	22,030	18,202	**12,035**	-33.9%	-45.4%
Toxicity and other safety testing including pharmacology					
Acute and sub-acute toxicity testing methods					
LD50, LC50	23,765	18,560	**17,156**	-7.6%	-27.8%
Other lethal methods	499	6,011	**2,376**	-60.5%	+376.2%
Non-lethal methods	21,947	14,401	**10,993**	-23.7%	-49.9%
Skin irritation/corrosion	3,998	2,793	**2,816**	+0.8%	-29.6%
Skin sensitisation	37,606	33,029	**29,365**	-11.1%	-21.9%
Eye irritation/corrosion	840	383	**334**	-12.8%	-60.2%
Repeated dose toxicity					
up to 28 days	43,484	34,860	**29,459**	-15.5%	-32.3%
29 - 90 days	22,938	21,249	**13,764**	-35.2%	-40%
> 90 days	12,888	11,240	**11,070**	-1.5%	-14.1%
Carcinogenicity	3,233	2,970	**4,044**	+36.2%	+25.1%
Genotoxicity	4,342	4,587	**4,095**	-10.7%	-5.7%
Reproductive toxicity	58,562	62,606	**84,259**	+34.6%	+43.9%
Developmental toxicity	63,271	96,807	**89,010**	-8.1%	+40.7%
Neurotoxicity	4,273	1,489	**1,951**	+31%	-54.3%
Kinetics	62,325	46,250	**37,896**	-18.1%	-39.2%
Pharmaco-dynamics (incl safety pharmacology)	78,934	32,043	**24,118**	-24.7%	-69.4%
Phototoxicity	519	600	**647**	+7.8%	+24.7%
Ecotoxicity					
Acute toxicity	54,115	36,347	**32,711**	-10%	-39.6%
Chronic toxicity	26,824	37,086	**37,928**	+2.3%	+41.4%
Reproductive ecotoxicity	240	710	**901**	+26.9%	+275.4%
Endocrine activity	790	8,286	**1,011**	-87.8%	+28%
Bioaccumulation	3,511	2,027	**1,188**	-41.4%	-66.2%

Other ecotoxicity	3,339	1,850	**1,685**	-8.9%	-49.5%
Safety testing in food and feed area	41,208	10,775	**6,145**	-43%	-85.1%
Target animal safety	6,290	8,343	**4,295**	-48.5%	-31.7%
Combined end-points	0	332	**4,793**	+1343.7%	NA
Other toxicity/safety testing	4,145	4,297	**3,132**	-27.1%	-24.4%
Other efficacy and tolerance testing					
Other efficacy and tolerance testing	96,803	32,101	**26,071**	-18.8%	-73.1%
Routine production					
Blood-based products	211,522	113,959	**66,954**	-41.2%	-68.3%
Monoclonal antibody by mouse ascites method	54,941	49,309	**35,981**	-27%	-34.5%
Monoclonal and polyclonal antibodies (excluding ascites method)	0	184,830	**220,916**	+19.5%	NA
Other product types	87,746	129,534	**100,440**	-22.5%	+14.5%
Other					
Protection of the natural environment in the interests of the health or welfare of human beings or animals	119,297	147,460	**152,642**	+3.5%	+28%
Preservation of species	83,683	496,041	**569,275**	+14.8%	+580.3%
Higher education	0	42,439	**39,458**	-7%	NA
Training for the acquisition, maintenance or improvement of vocational skills	0	102,839	**97,448**	-5.2%	NA
Higher education or training for the acquisition, maintenance or improvement of vocational skills	165,110	0	**0**	NA	-100%
Forensic enquiries	273	177	**109**	-38.4%	-60.1%
Total	**8,979,081**	**8,477,845**	**8,076,595**	**-4.7%**	**-10.1%**

Table 11: Total number of uses of animals by detailed purpose categories for 2018, 2022 and 2023

II.2.2.1. Research-related uses

Research-related uses are broken down by basic research on the one hand and translational and applied research on the other. The total number of uses for research purposes decreased by 6% between 2022 and 2023 (by 10.8% in basic research and by 1% in translational and applied research) (Table 8).

Looking at the breakdown of uses of animals for research in 2023 (Table 11), the five most important categories of uses in 2023 were for research into the nervous system, animal diseases and disorders, animal nutrition, animal welfare and the immune system.

If research purposes are classified by research centred on human, animal or other research[5], research centred on animals fluctuated significantly between 2018 and 2023 while research focusing on humans remained stable (despite a slight decrease in 2020 due to the COVID-19 pandemic) (Table 12).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Human research	4,341,800 (65.5%)	4,108,635 (67.3%)	**3,695,102 (64.4%)**	-10.1%	-14.9%
Animal research	2,063,380 (31.1%)	1,887,051 (30.9%)	**1,976,213 (34.4%)**	+4.7%	-4.2%
Other research	228,513 (3.4%)	111,959 (1.8%)	**69,255 (1.2%)**	-38.1%	-69.7%
Total	**6,633,693 (100%)**	**6,107,645 (100%)**	**5,740,570 (100%)**	**-6%**	**-13.5%**

Table 12: Total number of uses for research purposes in 2018, 2022 and 2023

5 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), with the remaining categories being considered as human-centred research.

The main species used in research centred on humans are mice (81.3%), fish (zebra fish in particular) (9.5%) and rats (6.6%). The main species used in research focusing on animals are fish (81.7%), birds (11.3%) and other mammals (farm animals in particular) (3.8%).

II.2.2.1.1 Basic research

Animal use for basic-research purposes decreased significantly in 2023 compared to 2022 by 10.8%, going below three million uses for the first time ever. This is a 30.3% reduction in animal use since 2018, confirming the long-term downward trend, despite a slight increase in 2022.

The three main areas of basic research using the most animals were research into the nervous system, the immune system and oncology, which together accounted for over half of all animal uses in basic research (Table 11).

In 2023, the main reductions in animal use compared to 2022 were for research related to the sensory organs (skin, eyes and ears) (down by 34.4%) and the gastrointestinal system including the liver (-23.4%). The highest increase was for urogenital/reproductive systems (+35%) and developmental biology (+10.9%) (Table11).

Animal use for 'other basic research' decreased once more in 2023 (down by 53.8%) confirming the general trend (-85.9% compared to 2018) and indicating more precise reporting by Member States. This category includes, for example, basic research on bioaccumulation, parasitology or hypoxia tolerance.

II.2.2.1.2. Translational and applied research

In 2023, translational and applied research was the main category for uses of animals (36.7% of all uses), accounting for 2.96 million uses in total, broadly unchanged since 2022 (-1%).

Research into animal diseases and disorders, animal nutrition and animal welfare still represented more than half of the uses in 2023. While uses for research into animal welfare decreased significantly (by 40.8%), uses in animal nutrition (up by 77.8%) and animal diseases and disorders (up by 53%) increased, the opposite of 2022, showing major fluctuations in the number of uses in these categories from one year to the next. Translational and applied research uses for animal-centred research warrant future examination to understand the reasons behind the substantial numbers of animals required and the major annual fluctuations for these uses.

Between 2022 and 2023, animal use for research into the study of human disorders decreased by 7.1%. There was an increase in uses for research into human urogenital/reproductive disorders (up 3.6%), confirming a longer-term trend with an increase of 76.9% between 2023 and 2018. But since 2018, there has been a decrease in use for research into human sensory organ disorders (skin, eyes and ears) (by 39.9%), human cardiovascular disorders (by 35.9%), human respiratory disorders (by 32.5%) and human immune disorders (by 30.9%) (Table11).

Animal use for 'other human disorders' decreased by 22.7% compared to 2022, which includes studies related to drug metabolism and pharmacokinetics or radiation studies, for example.

II.2.2.2. Uses of animals for regulatory purposes

Regulatory uses cover the use of animals in procedures to meet regulatory requirements, i.e. 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 planned but ultimately not made, e.g. because these

products/substances were deemed unsuitable for the market by the developer and thus failed to reach the end of the development process.

In 2023, regulatory uses accounted for 1.05 million uses. The total number of uses for regulatory purposes decreased by only 4.6% compared to 2022, but by a remarkable 35.1% compared to 2018.

Of these uses, 54.1% were related to quality control (including batch safety and potency testing), 43.4% were related to toxicity and other safety testing including pharmacology, and the remainder (2.5%) were for other efficacy and tolerance testing. Animal use in all these three categories has decreased since 2018 (Table 13).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Quality control (incl batch safety and potency testing)	942,127 (58.1%)	571,580 (51.8%)	**569,589 (54.1%)**	-0.3%	-39.5%
Toxicity and other safety testing including pharmacology	583,886 (36%)	499,931 (45.3%)	**457,142 (43.4%)**	-8.6%	-21.7%
Other efficacy and tolerance testing	96,803 (6%)	32,101 (2.9%)	**26,071 (2.5%)**	-18.8%	-73.1%
Total	**1,622,816 (100%)**	**1,103,612 (100%)**	**1,052,802 (100%)**	**-4.6%**	**-35.1%**

Table 13: Regulatory uses by type of research between 2018 and 2023

The main species used for regulatory purposes are mice (33.9%), rats (27.2%), 'other mammals' (16.7%), birds (11.3%) and fish (10.7%).

The 'other efficacy and tolerance testing' category covers uses that do not fall under quality control or to toxicity testing. For example, it includes testing the efficacy (immunogenicity) of human and veterinary vaccines.

II.2.2.2.1. Details of the regulatory uses

II.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 for a product (and its constituents) such as vaccines. It also includes any checks carried out during the manufacturing process for registration purposes, to meet any other national or international regulatory requirements or to satisfy the in-house policy of the manufacturer.

Quality-control-related uses amounted to 569 589 uses in 2023. Most of these uses, 454 749, were related to batch potency testing (Table 11) of which 18.3% were severe (Figure 8). Animal use in this specific category has been decreasing since 2015 and has now stabilised, with a drop of only 0.3% compared to 2022.

Animal use for 'other quality controls' decreased in 2023 compared to 2022 (by 33.9%). This category includes, for example, pathogenicity tests and product purity or potency.

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Austria	1,923 (6.4%)	0 (0%)	**0 (0%)**	NA	-100%
Bulgaria	132 (0.4%)	1,800 (9.4%)	**750 (4.2%)**	-58.3%	+468.2%
Czechia	53 (0.2%)	0 (0%)	**0 (0%)**	NA	-100%
France	9,578 (32.1%)	7,145 (37.3%)	**5,161 (29%)**	-27.8%	-46.1%
Germany	6,291 (21.1%)	1,078 (5.6%)	**695 (3.9%)**	-35.5%	-89%
Greece	0 (0%)	12 (0.1%)	**0 (0%)**	-100%	NA
Hungary	20 (0.1%)	0 (0%)	**0 (0%)**	NA	-100%
Ireland	145 (0.5%)	966 (5%)	**1,834 (10.3%)**	+89.9%	+1164.8%
Italy	2,243 (7.5%)	1,518 (7.9%)	**1,784 (10%)**	+17.5%	-20.5%
Poland	192 (0.6%)	147 (0.8%)	**170 (1%)**	+15.6%	-11.5%
Romania	372 (1.2%)	0 (0%)	**18 (0.1%)**	NA	-95.2%
Slovenia	64 (0.2%)	73 (0.4%)	**64 (0.4%)**	-12.3%	0%
Spain	8,802 (29.5%)	6,429 (33.5%)	**7,294 (41%)**	+13.5%	-17.1%

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Total	29,815 (100%)	19,168 (100%)	17,770 (100%)	-7.3%	-40.4%

Table 14: Number of uses for pyrogenicity testing per country in 2018, 2022 and 2023

In 2023, pyrogenicity testing, for which alternative methods (applicable to most use cases) are available, continued to decrease (by 7.3% compared to 2022 and by 40.4% compared to 2018). In 2023, these uses were reported mainly by six countries carrying out 98.5% of all pyrogenicity testing, i.e. Spain (41%), France (29%), Ireland (10.3%), Italy (10%), Bulgaria (4.2%) and Germany (3.9%) (Table 14).

An increase in the use of rabbits in 2023 in Ireland was linked to the validation of non-animal alternatives for detecting pyrogenic contaminants, in preparation for replacing the current rabbit pyrogen test.

This general downward trend applies to most major reporting countries, with France and Germany showing a steady year-on-year reduction since 2019. Austria reported its last use of animals for pyrogenicity testing in 2018, Czechia and Hungary in 2020 (Figure 10).

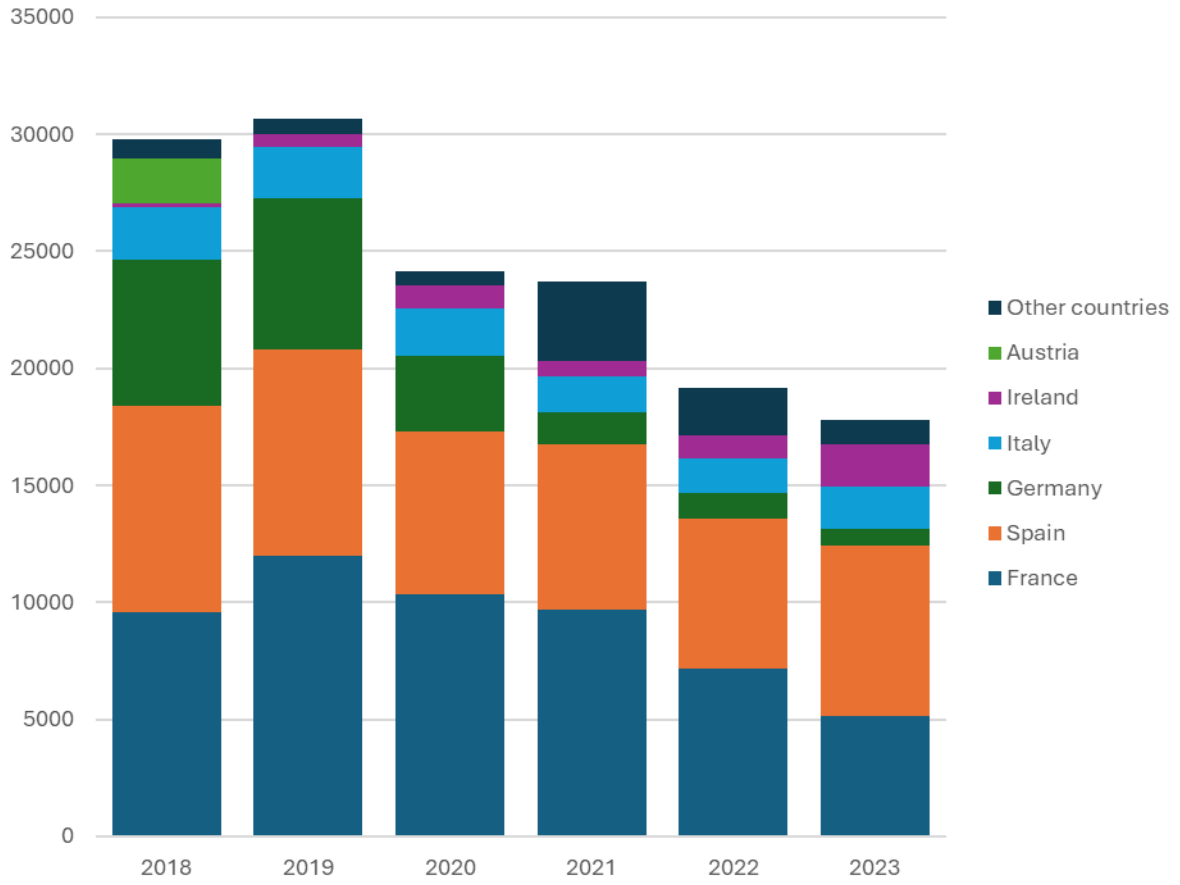

Figure 10: Trends in use of animals for pyrogenicity testing per country between 2018 and 2023

II.2.2.2.1.2. Toxicity and other safety testing including pharmacology

Toxicity and other safety testing (including the safety evaluation of products and devices for human medicine and dentistry, and veterinary medicine) covers studies carried out on any product or substance to assess 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 amounted to 457 142 uses of animals in 2023, which corresponded to 5.7% of all uses of animals. Most cases were related to developmental toxicity, reproductive toxicity, kinetics and acute or chronic toxicity for ecotoxicity testing.

Compared to 2022, the total number of uses for toxicity and other safety testing including pharmacology decreased (-8.6%), reflecting a continuous downward trend since 2018 (-21.7%) (Table 11).

II.2.2.2.2. Legislative aspects of regulatory uses

In 2023, most animal uses to meet regulatory requirements set by specific sector legislation were due to the placing on the market of medicinal products for humans (47.2%), veterinary medicinal products (26.4%) and industrial chemicals (16.6%) (Figure 11).

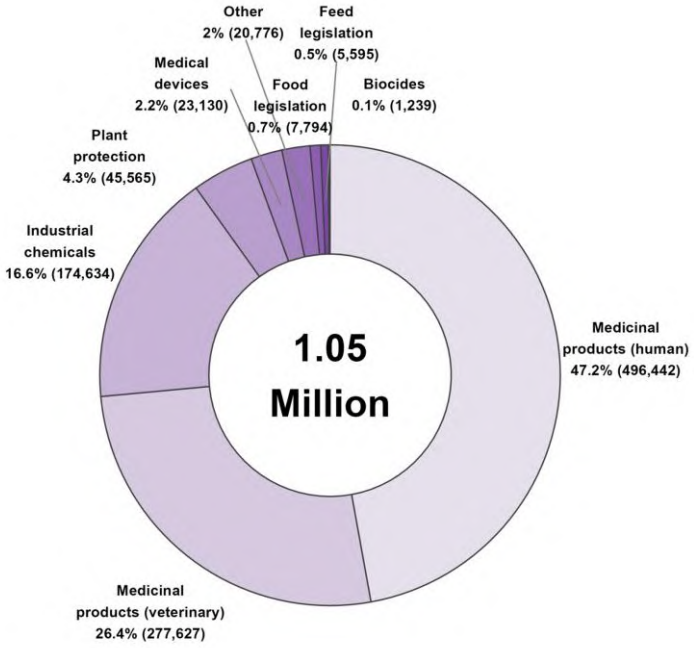

Figure 11: Regulatory uses by type of legislation in 2023

Between 2018 and 2023, animal use for regulatory purposes has constantly decreased (by 35.1%), mainly due to a decrease in uses for legislation on medicinal products for human use (down by 52%). Over the same period, animal use also decreased for food legislation purposes, including food contact material (by 81.3%) and 'other legislation' (by 53.2%) (Table 15).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Legislation on medicinal products for human use	1,034,432 (63.7%)	515,344 (46.7%)	**496,442 (47.2%)**	-3.7%	-52%
Legislation on medicinal products for veterinary use and their residues	265,949 (16.4%)	305,530 (27.7%)	**277,627 (26.4%)**	-9.1%	+4.4%
Medical devices legislation	52,335 (3.2%)	48,110 (4.4%)	**45,565 (4.3%)**	-5.3%	-12.9%

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Industrial chemicals legislation	123,837 (7.6%)	151,932 (13.8%)	**174,634 (16.6%)**	+14.9%	+41%
Plant protection product legislation	46,189 (2.8%)	35,895 (3.3%)	**20,776 (2%)**	-42.1%	-55%
Biocides legislation	1,788 (0.1%)	4,069 (0.4%)	**1,239 (0.1%)**	-69.6%	-30.7%
Food legislation including food contact material	41,641 (2.6%)	8,141 (0.7%)	**7,794 (0.7%)**	-4.3%	-81.3%
Feed legislation including legislation for the safety of target animals, workers and environment	7,200 (0.4%)	8,744 (0.8%)	**5,595 (0.5%)**	-36%	-22.3%
Other legislation	49,445 (3%)	25,847 (2.3%)	**23,130 (2.2%)**	-10.5%	-53.2%
Total	**1,622,816 (100%)**	**1,103,612 (100%)**	**1,052,802 (100%)**	**-4.6%**	**-35.1%**

Table 15: Regulatory uses by type of legislation between 2018 and 2023

In 2023, uses for industrial chemicals legislation continued to increase, rising by 14.9% (Table 15). This increase does not seem to stem from additional information requirements or an increase in chemicals coming to market. It may be due to the 2018 Commission-ECHA Joint Evaluation action plan (see Progress in evaluation - ECHA)[6]. This has led to testing requests (issued over the last five years) for dossiers submitted under REACH some time ago, but found to be non-compliant, i.e. not containing the information required or containing changes to testing that were not accepted (Figure 12).

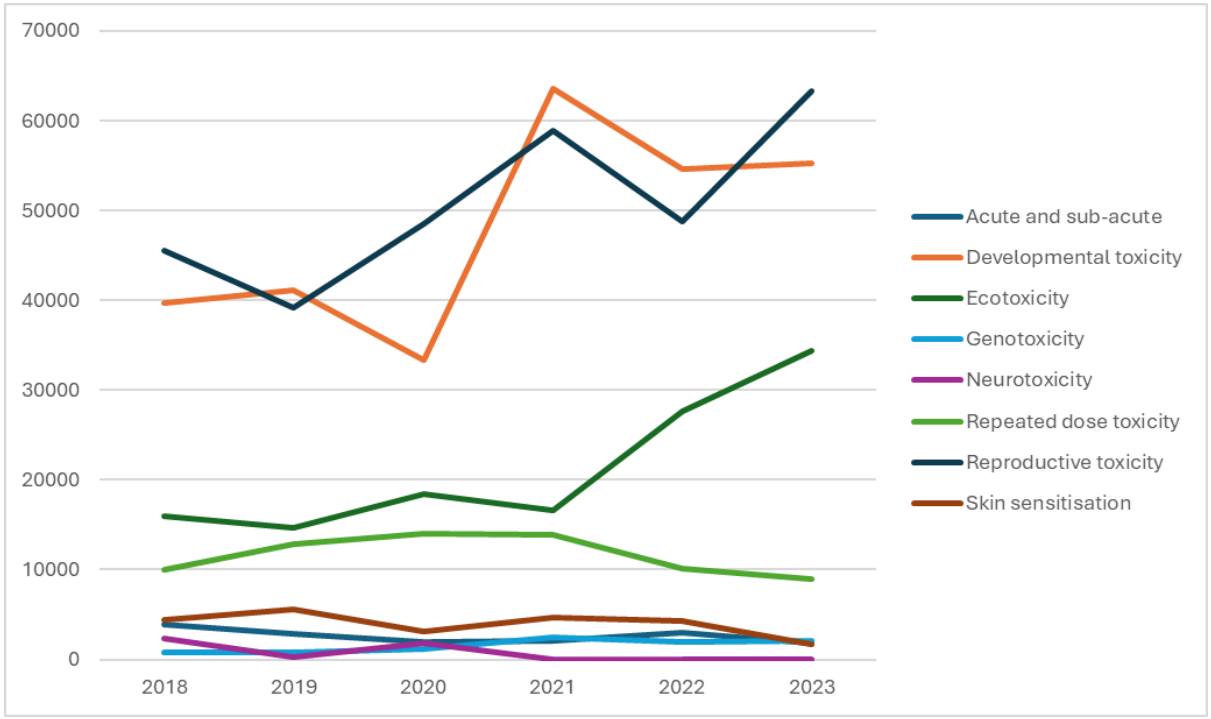

Figure 12: Trend in uses of animals for industrial chemicals legislation 2018-2023

[6] Progress in dossier evaluation.

In 2023, most regulatory uses were to meet regulatory requirements laid down by the Union (95.4%). Non-Union requirements accounted for 2.6% of uses and national requirements for 2%, proportions that have remained stable over the last five years.

The sub-category for legislation meeting Union requirements also includes any requirements for which international harmonisation has been achieved, such as for testing to OECD, ICH[7] and VICH[8] standards. Harmonisation testing requirements at global level is of utmost importance given the goal to avoid unnecessary duplication of testing.

The distribution of testing types (quality control; toxicity and other safety testing; other efficacy and tolerance testing) across the different areas of legislation appears to have remained stable from one year to the next.

II.2.2.3. Routine production uses

Animal use for routine production includes uses for the production of antibodies and blood products, including polyclonal antisera, using established methods. The main species used for this purpose are rabbits (65%), rodents (24.8%) and birds (8.9%).

In 2023, there were about 424 291 animal uses for routine production, representing 5.3% of all uses of animals in the Union and a 19.8% decrease since 2018 (Table 8). More than half the uses for routine production were for the production of (i) mono- and polyclonal antibodies (excluding the mouse ascites method) and (ii) monoclonal antibodies using the mouse ascites method. Animal use for (i) decreased by 27% but increased for (ii) by 19.5% compared to 2022. Animal use for blood-based products decreased by 41.2% (Table11). This can be explained by improved reporting from Member States following the 2021 amendment to these reporting categories.

While blood-based products involved only mild or moderate levels of severity, monoclonal antibody production using the mouse ascites method involved mostly severe uses (95.1%). This is one of the most severe procedures, the use of which has not yet fallen steadily since 2018 (Figure 13). In total, only four Member States reported using the mouse ascites method to produce monoclonal antibodies, with France making up the bulk of this at 98.5%. (Figure13).

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

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

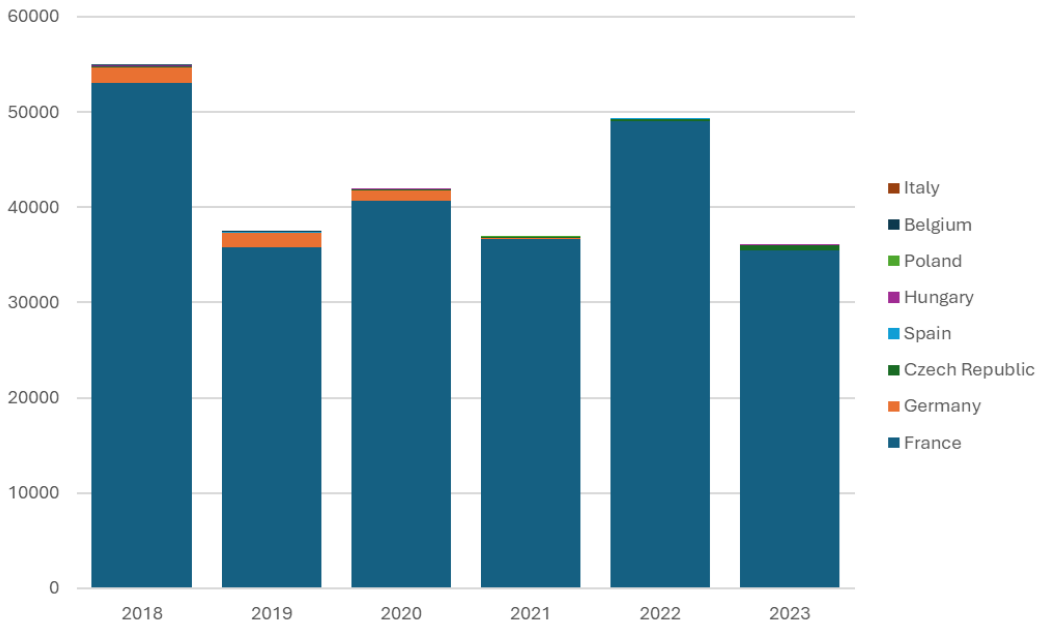

Figure 13: Trend in uses of animals for the production of monoclonal antibodies by country 2018-2023

In 2023, 12 Member States reported animal use for the production of mono- and polyclonal antibodies (excluding the mouse ascites method) (down from 14 in 2022).

Animal use for 'other product types', which accounted for 27.6% of routine production uses, were mostly related to antigenic, protein or vaccine production.

II.2.2.4. Other types of use

In 2023, the last five categories of use reported under the Directive covered 858 932 uses, up 8.9% from 2022 due to an 14.8% increase in uses for the preservation of species (Table 8).

Looking at the species used for these purposes, two categories emerged: fish species were mainly used for the protection of the natural environment in the interests of the health or welfare of human beings or animals (80.7%) and the preservation of species (98.0%). Mice and rates were more often used for higher education and training for the acquisition, maintenance or improvement of vocational skills (44.9% and 58.9% for mice), (22.8% and 20.7% for rats).

In 2023, 569 275 animals uses were recorded for the preservation of species, the biggest category of the remaining types of use, representing more than half of 'other' uses. Forensic inquiry uses accounted for only about a hundred animal uses (Table 11).

II.2.3. Information on reuse and genetic status of animals

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

II.2.3.1. Reuse

In line with the principle of the Three Rs (Replacement, Reduction, Refinement), the total number of animals used in procedures can be reduced by performing procedures on animals more than once. However, reuse should only be permitted when it does not result in poor animal welfare, and it should be evaluated on a case-by-case basis. Under the Directive, reuse of

animals in procedures is permitted only if the following are taken into account: the actual level of severity the animal has experienced in a previous procedure, and the health and well-being of the animal, considering the lifetime experience of the individual animal. Reuse cannot be authorised for a procedure in which the animal may reach a severe level of pain, suffering or distress. An animal may be reused following a severe procedure only in exceptional circumstances and after having undergone a veterinary examination.

In 2023, the proportion of reuse was 1.3%, a slight increase compared to 2022 both in absolute numbers (up 10.7%) and as a proportion (by 0.2 percentage points) (Table 16).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
No	8,822,404 (98.3%)	8,385,397 (98.9%)	7,974,226 (98.7%)	-4.9%	-9.6%
Yes	156,677 (1.7%)	92,448 (1.1%)	102,369 (1.3%)	+10.7%	-34.7%
Total	**8,979,081 (100%)**	**8,477,845 (100%)**	**8,076,595 (100%)**	**-4.7%**	**-10.1%**

Table 16: Reuse of animals used for research, testing, routine production and educational purposes

Procedures requiring the reuse of animals are mostly related to regulatory use: skin irritation/corrosion (49.2%), pyrogenicity testing (36.5%), training for the acquisition, maintenance or improvement of vocational skills (9.9%), higher education (9.5%), eye irritation/corrosion (7.8%) and pharmaco-dynamics (including safety pharmacology) (7.8%). In absolute numbers, the main procedures requiring the reuse of animals were animal welfare (10 754); quality: batch potency testing (9 850); training for the acquisition, maintenance or improvement of vocational skills (9 606); ethology / animal behaviour / animal biology (7 019) and quality: pyrogenicity testing (6 481).

Looking at the types of species, non-human primates are reused more often than other species: squirrel monkeys (87.5%), rhesus monkeys (39.1%), cynomolgus monkeys (24.8%) and baboons (19.0%); but the absolute numbers are low. Other animals frequently reused are large mammal species such as cats (52.5%), goats (39.3%), dogs (36.7%), horses, donkeys and cross-breeds (26.1%), cattle (17.1%) or sheep (14.9%), in larger absolute numbers. Xenopus (20.8%) and reptiles (20%) are also often reused. In absolute numbers, the main species reused for scientific purposes in 2023 were mice (33 557), rabbits (11 962), other fish (10 799), salmon, trout, chars and graylings (7 828), rats (7 301) and dogs (4 848).

	2018	2022	2023
Mild [up to and including]	107,781 (68.8%)	62,866 (68%)	**75,623 (73.9%)**
Moderate	36,053 (23%)	26,094 (28.2%)	**23,461 (22.9%)**
Non-recovery	12,762 (8.1%)	2,826 (3.1%)	**2,959 (2.9%)**
Severe	81 (0.1%)	662 (0.7%)	**326 (0.3%)**
Total	**156,677 (100%)**	**92,448 (100%)**	**102,369 (100%)**

Table 17: Severity classification of reuse procedures

Under the Directive, reuse of an animal is not allowed in a procedure classified prospectively as severe. In 2023, for most cases of reuse, the actual reported severities were mild (73.9%) or moderate (22.9%) (Table 17).

However, in some cases, even if the procedure has been prospectively classified in a lower severity category, an individual animal may reach the ‘severe’ category due to unforeseen events occurring during the procedure. Only a very small number of such cases (0.3%) was reported, with 326 such cases in 2023, down from 662 in 2022.

These 326 uses should be investigated by the authorities to avoid the recurrence. If such events do recur, this may suggest a need to revise the prospective classification in the future.

II.2.3.2. Use of genetically altered animals

Some of the animals used in procedures for the purposes of research, testing, routine production and education are genetically altered. This section outlines the types of genetic alteration reported. A welfare assessment must be carried out 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 the 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 gene alteration) or additional care beyond that required for conventional animals to maintain their health and well-being.

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Genetically altered with a harmful phenotype	275,660 (3.1%)	368,197 (4.3%)	**325,818 (4%)**	-11.5%	+18.2%
Genetically altered without a harmful phenotype	1,518,800 (16.9%)	1,735,560 (20.5%)	**1,481,275 (18.3%)**	-14.7%	-2.5%
Not genetically altered	7,184,621 (80%)	6,374,088 (75.2%)	**6,269,502 (77.6%)**	-1.6%	-12.7%
Total	**8,979,081 (100%)**	**8,477,845 (100%)**	**8,076,595 (100%)**	**-4.7%**	**-10.1%**

Table 18: Genetic status of animals used in 2023

In 2023, the uses of genetically altered animals for scientific purposes decreased to 22.3% (2.4 percentage points lower than in 2022) representing 1.81 million uses. Of these animals, 18% carried a harmful phenotype (Table 18; Figure 14).

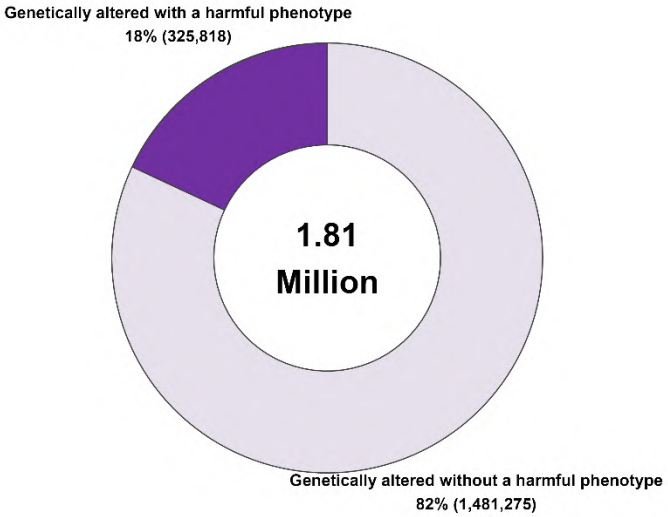

Figure 14: Uses of animals by type of genetic alteration in 2023

Amongst the species that have been genetically altered, uses of mice accounted for the highest numbers, followed by zebra fish and rats. Although mice are the largest group of animals being genetically altered, proportionally they are not. 57.7% of zebra fish used are genetically altered, followed by 42.8% of mice used, while only 3.5% of rats were genetically altered and used in procedures for the purposes of research, testing and routine production in 2023 (Table 19).

In terms of trends since 2018, the proportion of genetically altered zebra fish has increased (by 15.2 percentage points), while the proportion of genetically altered rabbits has decreased (by 3.5 percentage points).

	2018	2019	2020	2021	2022	2023
Mice	36.1%	38.9%	40.6%	41.7%	45.9%	**42.8%**
Rats	3.5%	3.2%	3.4%	3.7%	4.3%	**3.5%**
Hamsters (Syrian)	2.8%	5.3%	0%	0.8%	2.5%	**1.9%**
Other rodents	0%	0.1%	0%	0%	0.2%	**0.2%**
Rabbits	7.5%	6.2%	5.1%	5.4%	5%	**4%**
Dogs	0.4%	0.2%	0.1%	0.1%	0.1%	**0.1%**
Ferrets	0%	0.7%	3%	0%	1.8%	**2.3%**
Pigs	0.6%	0.8%	0.8%	1%	1.1%	**1.3%**
Sheep	0%	0%	0%	0.3%	0%	**0%**
Prosimians	0%	0%	0%	23.4%	0%	**0%**
Domestic fowl	0%	0%	0%	0.1%	0.2%	**0.1%**
Xenopus	20.2%	8.4%	7.4%	2.6%	9.6%	**19.1%**
Other amphibians	11.2%	18.2%	27.4%	32%	20.4%	**24.2%**
Zebra fish	42.5%	49.1%	52.4%	49.2%	51.3%	**57.7%**
Other fish	0.2%	0.2%	0.3%	0.7%	0.6%	**0.9%**

Table 19: Genetically altered species used between 2018 and 2023

Genetically altered animals are used almost exclusively for research purposes. In 2023, the proportion of genetically altered animals was highest in basic research into the musculoskeletal system (72%), followed by sensory organs (68.9%), the cardiovascular blood and lymphatic system (66%), developmental biology (59.5%), human musculoskeletal disorders (56.5%), multisystemic (53.6%), the nervous system (52.7%), the immune system (51.2%), the endocrine system/metabolism (51%) and oncology (50.7%).

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

Under the Directive, Member States are also required to report on the animals used in procedures for the creation of new genetically altered animal lines, and on the maintenance of colonies of established genetically altered animal lines to support research needs in the Union.

Reuse under both of these categories remains extremely low with no year-on-year fluctuations and is therefore not further analysed in this report. Detailed data are available in the ALURES statistical database.

II.3.1. All uses of animals for the creation of new genetically altered animal lines

In 2023, 330 458 animals were used for the first time for the creation of new genetically altered animal lines (Table 20); a decrease of 13.6% between 2018 and 2023.

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Mammals					
Rodents					
Mice	274,354	250,551	**246,554**	-1.6%	-10.1%
Rats	6,168	1,910	**1,763**	-7.7%	-71.4%
Guinea Pigs	0	0	**0**	NA	NA
Hamsters (Syrian)	89	120	**0**	-100%	-100%
Other rodents	0	0	**0**	NA	NA
Rabbits					
Rabbits	324	216	**166**	-23.1%	-48.8%
Carnivores					
Dogs	0	0	**0**	NA	NA
Ferrets	4	0	**0**	NA	-100%
Other carnivores	0	0	**0**	NA	NA
Farm animals					
Horses, donkeys and cross-breeds	0	0	**0**	NA	NA
Pigs	62	279	**220**	-21.1%	+254.8%
Goats	0	38	**87**	+128.9%	NA
Sheep	0	6	**9**	+50%	NA
Cattle	0	0	**0**	NA	NA
Non-human primates					
Marmoset and tamarins	10	8	**11**	+37.5%	+10%
Rhesus monkey	0	0	**14**	NA	NA
Other mammals					
Other mammals	70	6	**0**	-100%	-100%
Birds					
Domestic fowl	100	612	**790**	+29.1%	+690%
Other birds	0	0	**0**	NA	NA
Reptiles					
Reptiles	0	0	**0**	NA	NA
Amphibians					
Xenopus	0	4,160	**5,515**	+32.6%	NA
Other amphibians	100	1,324	**33**	-97.5%	-67%
Fish					
Zebra fish	84,996	82,864	**71,655**	-13.5%	-15.7%
Other fish	16,087	3,780	**1,892**	-49.9%	-88.2%
Salmon, trout, chars and graylings	0	3,232	**1,749**	-45.9%	NA
Totals					
Total	**382,364**	**349,106**	**330,458**	**-5.3%**	**-13.6%**

Table 20: Uses of animals for the creation of new genetically altered animal lines by species in 2018, 2022 and 2023

The creation of a new genetically altered animal line must be reported under the research purpose category for which the line is being created. The reporting covers all animals carrying the genetic alteration. In addition, animals used for superovulation, vasectomy and embryo implantation are also reported on (they may or may not themselves be genetically altered). Genetically normal animals (wild-type offspring) produced as a result of the creation of a new genetically altered line are excluded from the annual statistics.

Counting all uses, the main species that were used for the creation of new genetically altered animal lines were mice (74.6%) and zebra fish (21.7%). Other species included, in very small numbers, other species of fish, rats, salmon, trout, chars and graylings.

II.3.1.1. Creation of new genetically altered animal lines by genetic status

Animals that are not genetically altered but are reported under the category ‘creation of a new genetically altered animal line’ include, for example, genetically normal parent animals or any of the offspring not carrying the genetic alteration but which has been genotyped (tissue

sampled) using an invasive method. Of those that were genetically altered, 63.5% were of a non-harmful phenotype (Figure 15).

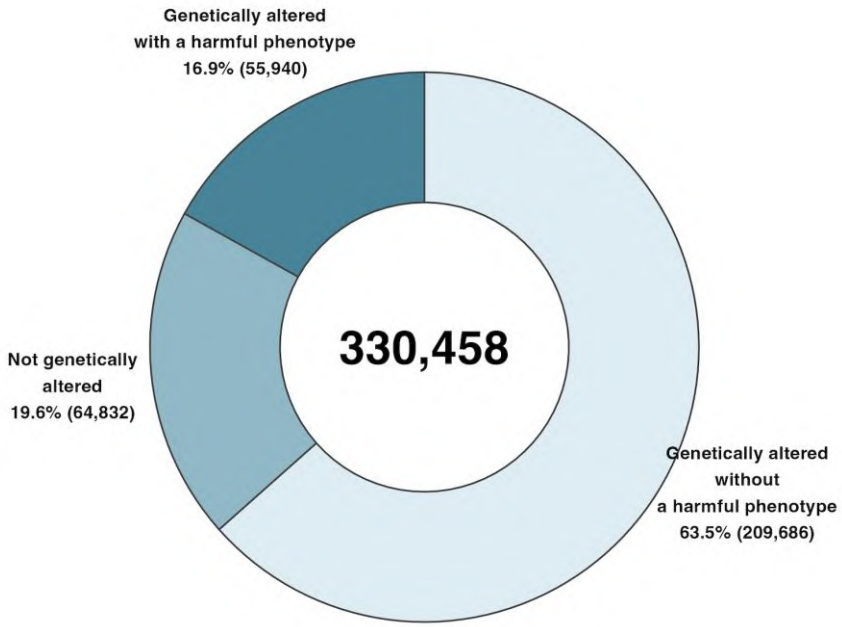

Figure 15: Creation of new genetically altered animal lines – genetic types of animals used in 2023

II.3.1.2. Creation of new genetically altered animal lines for scientific purposes

The creation of new genetic lines is only carried out for research purposes. In 2023, 330 458 uses (first and any subsequent reuses) were reported for the purposes of creating new genetically altered animal lines.

Of the new genetically altered lines, 89.8% were created for purposes falling under basic research. Table 21 below sets out all the sub-categories for both basic and translational and applied research.

In 2023, basic research covered uses for the nervous system (18.6%), oncology (13.4%) and multisystemic uses (13.2%). The most important sub-category under translational and applied research for which new genetically altered animal lines were created was human infectious disorders (4.7%).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Basic research					
Oncology	50,180 (13.1%)	29,298 (8.4%)	**44,233 (13.4%)**	**+51%**	-11.9%
Cardiovascular Blood and Lymphatic System	42,560 (11.1%)	38,889 (11.1%)	**32,195 (9.7%)**	**-17.2%**	-24.4%
Nervous System	63,676 (16.7%)	68,193 (19.5%)	**61,479 (18.6%)**	**-9.8%**	-3.5%
Respiratory System	510 (0.1%)	669 (0.2%)	**646 (0.2%)**	**-3.4%**	+26.7%
Gastrointestinal System including Liver	9,283 (2.4%)	13,808 (4%)	**6,866 (2.1%)**	**-50.3%**	-26%
Musculoskeletal System	11,068 (2.9%)	6,621 (1.9%)	**6,319 (1.9%)**	**-4.6%**	-42.9%
Immune System	33,529 (8.8%)	30,405 (8.7%)	**32,493 (9.8%)**	**+6.9%**	-3.1%
Urogenital/Reproductive System	22,100 (5.8%)	16,908 (4.8%)	**20,832 (6.3%)**	**+23.2%**	-5.7%
Sensory Organs (skin, eyes and ears)	10,957 (2.9%)	2,501 (0.7%)	**5,871 (1.8%)**	**+134.7%**	-46.4%
Endocrine System/Metabolism	17,086 (4.5%)	28,009 (8%)	**20,548 (6.2%)**	**-26.6%**	+20.3%
Developmental Biology	0 (0%)	18,827 (5.4%)	**19,541 (5.9%)**	**+3.8%**	NA
Multisystemic	59,131 (15.5%)	65,270 (18.7%)	**43,660 (13.2%)**	**-33.1%**	-26.2%
Ethology / Animal Behaviour /Animal Biology	2,388 (0.6%)	100 (0%)	**184 (0.1%)**	**+84%**	-92.3%
Other basic research	23,830 (6.2%)	3,280 (0.9%)	**2,016 (0.6%)**	**-38.5%**	-91.5%
Translational and applied research					
Human Cancer	3,221 (0.8%)	1,265 (0.4%)	1,843 (0.6%)	**+45.7%**	-42.8%
Human Infectious Disorders	1,062 (0.3%)	9,339 (2.7%)	15,674 (4.7%)	**+67.8%**	+1375.9%

	Human Cardiovascular Disorders	2,298 (0.6%)	1,610 (0.5%)	3,452 (1%)	**+114.4%**	+50.2%
	Human Nervous and Mental Disorders	3,156 (0.8%)	4,685 (1.3%)	3,539 (1.1%)	**-24.5%**	+12.1%
	Human Respiratory Disorders	608 (0.2%)	457 (0.1%)	201 (0.1%)	**-56%**	-66.9%
	Human Gastrointestinal Disorders including Liver	3,224 (0.8%)	876 (0.3%)	923 (0.3%)	**+5.4%**	-71.4%
	Human Musculoskeletal Disorders	317 (0.1%)	2,290 (0.7%)	1,075 (0.3%)	**-53.1%**	+239.1%
	Human Immune Disorders	429 (0.1%)	1,220 (0.3%)	1,071 (0.3%)	**-12.2%**	+149.7%
	Human Urogenital/Reproductive Disorders	171 (0%)	299 (0.1%)	304 (0.1%)	**+1.7%**	+77.8%
	Human Sensory Organ Disorders (skin, eyes and ears)	783 (0.2%)	3,088 (0.9%)	2,367 (0.7%)	**-23.3%**	+202.3%
	Human Endocrine/Metabolism Disorders	10,551 (2.8%)	880 (0.3%)	1,233 (0.4%)	**+40.1%**	-88.3%
	Other Human Disorders	820 (0.2%)	86 (0%)	149 (0%)	**+73.3%**	-81.8%
	Animal Diseases and Disorders	9,169 (2.4%)	163 (0%)	192 (0.1%)	**+17.8%**	-97.9%
	Animal Nutrition	0 (0%)	0 (0%)	92 (0%)	**NA**	NA
	Animal Welfare	223 (0.1%)	0 (0%)	56 (0%)	**NA**	-74.9%
	Diagnosis of diseases	0 (0%)	8 (0%)	878 (0.3%)	**+10875%**	NA
	Non-regulatory toxicology and ecotoxicology	34 (0%)	62 (0%)	526 (0.2%)	+748.4%	+1447.1%
Total		**382,364 (100%)**	**349,106 (100%)**	**330,458 (100%)**	**-5.3%**	**-13.6%**

Table 21: Uses of animals for the creation of new genetically altered animal lines by type of research between 2018 and 2023

II.3.1.3. Creation of new genetically altered animal lines by severity

The severities reported under the category of animal uses for the creation of new genetically altered animal lines include the impact of surgical techniques used during creation (embryo transfer, vasectomy), tissue sampling (using an invasive method for genotyping) and the effects expressed by the phenotype of the genetic alteration (Figure 16).

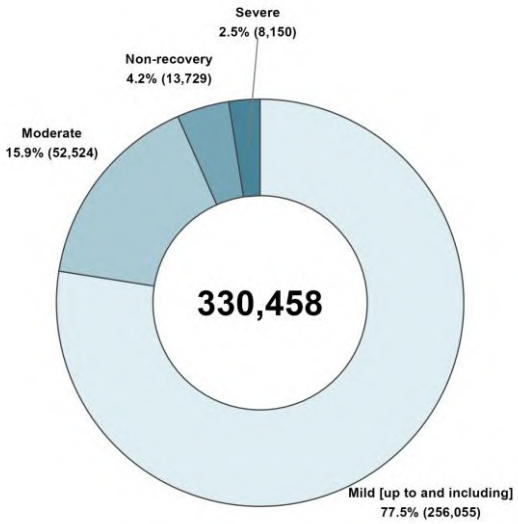

Figure 16: Uses of animals for the creation of new genetically altered animal lines by severities in 2023

II.3.2. All uses of animals for the maintenance of colonies of established genetically altered animal lines

This category covers 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 either of the following applies:

- the genotype has been confirmed using an invasive method (tissue sampling/genotyping), which was not carried out for the purposes of identification/marking of the animal, and the animal is killed without further use;
- the animals are of unsuitable genotype, confirmed using an invasive method, which was not carried out for the purposes of identification/ marking of the animal.

The reporting requirements for the maintenance of colonies of established genetically altered animal lines are particularly complex. To address this, the Commission, Member States and key stakeholder organisations developed a guidance document which was finalised in November 2021[9]. The translations into all Union languages were published in 2022, which should improve the consistency and accuracy of reporting.

II.3.2.1. Maintenance of colonies of established genetically altered animal lines by genetic status

In 2023, 666 555 uses were reported under the category of maintenance of colonies of established genetically altered animal lines. Animal use in this category increased by 29.7% since 2022. The reason for the increase seems to be the effort made by France to improve the accuracy of reporting in this category.

Amongst these uses, 86% were genetically altered without a harmful phenotype, 9.5% with a harmful phenotype and 4.9% without genetic alteration (Figure 17).

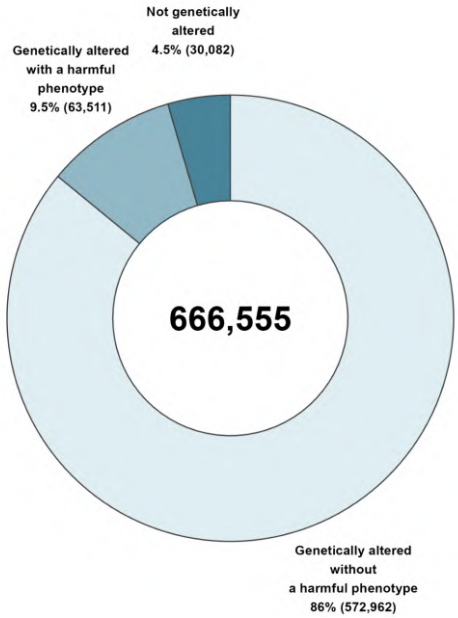

Figure 17: Genetic status of animals used for the maintenance of colonies of established genetically altered animal lines in 2023

[9] Guidance document on genetically altered animals

II.3.2.2. Maintenance of colonies of established genetically altered animal lines by severity

In 2023, the level of severity of 93.8% of animal uses was mild (up to and including) (Figure 18). Looking at Figure 17, showing that 86% of animals were of a non-harmful phenotype, the severity seems to be linked to the effects of tissue sampling (invasive genotyping). For those classified as having a harmful phenotype, the severity can be linked to the phenotype and invasive tissue sampling. If an animal is found dead and no informed decision can be made on the cause of and the events preceding death, it is reported as 'severe'.

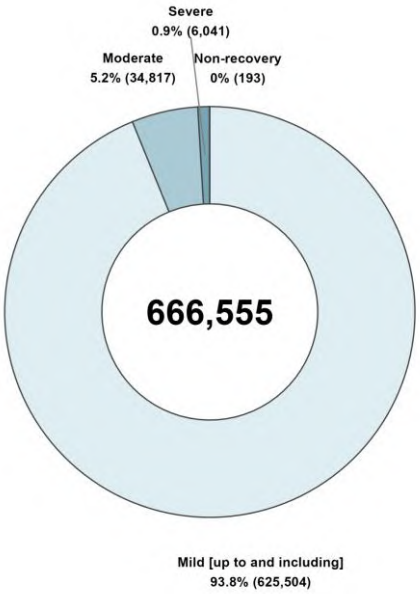

Figure 18: Uses of animals for the maintenance of colonies of genetically altered animal lines by severity in 2023

II.3.2.2. Maintenance of colonies of established genetically altered animal lines by species

Mice, rats and zebra fish are the most common genetically altered animals used for scientific purposes and are therefore also the main species used for the maintenance of colonies (Table 22).

	2018	2022	2023	Change 2023 vs 2022	Change 2023 vs 2018
Mammals					
Rodents					
Mice	518,180	463,034	**620,182**	+33.9%	+19.7%
Rats	3,827	10,744	**16,557**	+54.1%	+332.6%
Other rodents	0	41	**0**	-100%	NA
Carnivores					
Dogs	5	27	**27**	0%	+440%
Birds					
Domestic fowl	219	0	**0**	NA	-100%
Amphibians					
Xenopus	0	19	**0**	-100%	NA
Fish					
Zebra fish	7,840	39,189	**29,441**	-24.9%	+275.5%
Other fish	1,262	724	**348**	-51.9%	-72.4%
Totals					

Total	**531,333**	**513,778**	**666,555**	**+29.7%**	**+25.4%**

Table 22: Uses of animals for the maintenance of colonies of established genetically altered animal lines by species between 2018 and 2023

III. Member State narratives 2023

Member States submitted 2023 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 2023, and Norway.

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

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 2023 which can be found in this section of the EU summary report.

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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 2023 is 223 266 (2021: 218 244; 2022: 211 338) and is within the fluctuation range of the last 10 years (approx. +6 % or in absolute numbers an increase of 11 928 animals compared to 2022). The numbers include all uses (first and any subsequent use and 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.

200 596 animals were **used for the first time** for research, testing, routine production and education and training purposes which is an increase of 14 570 animals compared to 2022 (total number of animals used 186 026).

All uses of animals for research, testing, routine production and education and training purposes increased from 186 698 animals in 2022 to 201 585 animals, mainly mice and zebrafish, in 2023. Approx. 55% were classified as mild (up to and including) procedures. Severe procedures reduced from approx. 12% in 2021 to 8% in 2022 and to 4% in 2023.

Numbers of animals used for the **creation of new genetically altered animal lines** for the first time decreased (from 28 259 in 2021 to 18 648 in 2022) to 15 478 animals, mainly mice, xenopus and zebra fish, in 2023. Approx. 87% were classified as mild (up to and including) procedures.

A total number of 6 203 animal uses (only mice) are reported in 2023 for the **maintenance of genetically altered animal lines**. Approx. 85% were classified as mild (up to and including) and 15% as moderate procedures.

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

In AT significant annual fluctuations in different categories are observed which can be explained by the varying focus on different research areas and research activities:
In 2023 a decrease in **all uses of animals** is observed in "Translational and applied research" (from 71 012 animals used in 2021 to 75 152 in 2022 to 73 108 in 2023), mainly in the category "Human Cancer".

An increase in all uses of animals is observed in "Basic Research" (from 93 482 animals in 2022 to 107 051 animals in 2023), mainly in the category "Musculoskeletal System" (from 6 078 animals used in 2022 to 25 271 in 2023).

A decrease of animals used for the **creation of new genetically altered lines** was observed in the use of zebrafish (from 14 524 animals used in 2021 to 8 999 animals used in 2022 to 2 694 animals used in 2023). An increase of animals used for the **creation of new genetically altered lines** was observed in the use of xenopus (from 688 animals used in 2022 to 4 998 animals used in 2023). An increase of animals used for the creation of new genetically altered lines was observed in "Basic research" (from 27 868 animals used in 2021 to 18 636 in 2022 to 15 274 in 2023), mainly in the categories "Nervous System"

(from 784 animals in 2022 to 6 094 animals in 2023) and "Oncology" (from 1 756 animals used in 2022 to 3 396 in 2023). A decrease of animals used for the creation of new genetically altered lines was observed in "Basic research" in the category "Multisystemic" (from 10 181 animals in 2022 to 1 185 animals in 2023).
Since 2018 only mice are used for the **Maintenance of genetically altered animal lines** (a total number of 6 203 mice in 2023).

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

Approx. 55% of **all uses of animals** for research, testing, routine production and education and training purposes were classified as mild (up to and including) procedures. Severe procedures reduced from approx. 12% in 2021 to 8% in 2022 to 4% in 2023.

Approx. 86% of uses for the **creation of new genetically altered animal lines** were classified as mild (up to and including) procedures (compared to 93% in 2022).

Approx. 85% of uses for the **maintenance of genetically altered animal lines** (only mice) were classified as mild (up to and including) (70% in 2022) and 15% as moderate (30% in 2022) procedures. No severe uses were reported for the maintenance of genetically altered animal lines since 2020.

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

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

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

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

"Other birds" (total 1 257 animals) include i.a. Parus major (657), Cyanistes caeruleus (167), Passer domesticus (120), Sylvia atricapilla (113), Acrocephalus scirpaceus (43), "Other fish" (total 12 363 animals) include i.a. Chondrostoma nasus (3 000), Barbus barbus (1,475), Squalius cephalus (1 357), Oryzias latipes (908), Rutilus rutilus (881), and "Other amphibian" (total 5 899 animals) include mainly Ambystoma mexicanum (5 376).

(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:**

Belgium

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

The total number of animal procedures performed in 2023 includes:

- All uses of animals (including re-use) for research, testing, routine production, education, and training purposes (426 585);
- all uses of animals for the creation of new genetically altered animal lines (23 664) and
- all uses of animals for the maintenance of genetically altered animal lines (24 893).

In 2023, there were 475 142 animal procedures performed, a very slight increase (0.38%) compared to the 473 329 procedures performed in 2022.

All uses of animals for research, testing, routine production, education, and training purposes (426 585).

There was a slight decrease (-1.59%) in this category with 433 495 uses in 2022 and 426 585 in 2023.

Looking at the species used for research, testing, routine production, education and training, some figures stand out.

- The number of procedures involving zebra fish increased significantly by 61.49% from 2022 to 2023. This is mainly due to a specific user working on autonomous larvae since 2022. This user was working with zebra fish eggs (not included in the Directive 2010/63/EU) before 2022 but had to change the technique to use larvae.
- The use of rabbits and mice remained relatively stable with a slight decrease of 1.71% and 4.02% respectively.
- The use of rats showed a significant decrease of 14.50%, which is explained by a reduction in the number of animal procedures involving rats in the field of human nervous and mental disorders (translational and applied research) and in the field of cardiovascular blood and lymphatic system (basic research).
- Procedures on domestic fowl decreased sharply in 2023. The decrease of 29.08% is mainly due to a decrease in the number of animal procedures related to research on animal diseases and conditions.

Less commonly used species show more pronounced changes. However, because of their small numbers, these variations are not relevant when considering general trends.

Looking at the reuse of animals used for research, testing, routine production, education, and training, we notice an increase in the number of animals reused in 2023. The reuse rate was 0.66% in 2022 and 1.10% in 2023. In 2023, mice (37.77%) were the main species reused, followed by rats (16.83%) and fish (15.17%).

All uses of animals for the creation of new genetically altered animal lines (23 664).

While the total number of animals used to create new genetically altered (GA) lines in 2023 (23 664) was quite similar to the number of animals used in 2022 (23 732), a closer analysis of the species used reveals some differences.

- The number of procedures on zebra fish showed a significant increase of 28.69% in 2023, while the number of mice used decreased by 5.40% in 2023 compared to 2022. The largest increase in the use of zebra fish can be attributed to increased use in the field of developmental biology (basic research). There are only minor shifts in the other fields where zebra fish were used. In contrast, the largest decrease in the use of mice is mainly explained by a decrease in human nervous and mental disorders (basic research) and in endocrine system/metabolism (translational and applied research).
- For the first time since 2019, no hamsters were used to create new GA lines while rabbits were introduced into this category for the first time (24 procedures), marking a new trend in their use for creation. These rabbits were used exclusively for research in human immune disorders (translational and applied research).
- The number of rats and goats used to create new GA animal lines increased significantly in 2023, but the final figure only affects a small number of animals (281 rats and 70 goats).

All uses of animals for the maintenance of genetically altered animal lines (24 893).

In this category, the number of procedures increased from 16 102 in 2022 to 24 893 in 2023. This is an increase of 54.60%.
The number of mice, which represent 98.91% of all animals used for the maintenance of genetically altered animal lines, increased by 54.80% in 2023 compared to 2022 (2022: 15 906; 2023: 24 622). This significant increase can be explained by a more accurate reporting of data.

(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 remains more or less stable between 2022 and 2023. However, we note a significant increase (225.34 %) in the number of procedures in the area of endocrine system / metabolism (28 298 uses in 2023 compared to 8 698 uses in 2022), leading to 15.01% of all uses in this category. This is mainly due to an increase in projects and theses on this topic carried out in a laboratory working with zebra fish in 2023. In addition, this laboratory used autonomous zebra fish larvae in 2023, whereas it used eggs before, which are not required to be reported in the statistical data. The main areas of research in the basic research category in 2023, nonetheless, remain the immune system (21.93%), oncology (20.38%) and the nervous system (17.78%).

A decrease of 6.25% was observed in the ‘Translational and applied research’ category in 2023 (126 589 procedures) compared to 2022 (135 034 procedures). The largest decrease between 2022 and 2023 was observed in the area of animal diseases and disorders (-15 271 procedures, i.e. a decrease of 42.61%). This is partly due to a continuing reduction in the number of feed additive studies that were conducted (where the primary parameter is often feed conversion, which can be evaluated at group level and which requires several replicates of multiple animals to provide sufficient power). On the other hand, a significant increase (42.04%) was observed for human cancer studies, which increased from 17 608

uses to 25 010 uses. More animal procedures were conducted in this area than last year due to an increase in the number of studies aimed at the local administration (intratumoral) of both radiation and chemotherapy in combination with immunotherapy.

The category 'Regulatory use and routine production' increased by only 2.69% between 2022 and 2023 (from 99 191 uses to 101 856 uses). However, this category always shows fluctuations over the years. This is mainly related to the requirement to carry out quality control in the area of regulatory use or not. In the area of routine production, there was a significant shift in the categories of use. Whereas in 2022 62 762 uses were reported for blood-based products, in 2023 only 159 were reported. On the other hand, the category of monoclonal and polyclonal antibody production showed the opposite movement, with 169 uses in 2022 compared to 61 621 in 2023. This is mainly due to a reporting error in 2021 and 2022 where rabbits were reported in the category blood based products instead of monoclonal and polyclonal antibodies (excluding ascites). Prior to 2021, no distinction was made between these two categories and animals used for the production of monoclonal or polyclonal antibodies (excluding the ascites method) had to be reported under the category blood based products.

The category 'Protection of the natural environment in the interests of the health or welfare of human beings or animals' decreased by 80.58% between 2022 and 2023 (from 309 uses to 60 uses). In 2023, 60 *Lithobates catesbeianus* were used to improve control measures for invasive American bullfrog populations using sterile individuals.

The category 'Preservation of species' continues to increase from 2020 onwards. There is an increase of 12.71% between 2022 and 2023. In 2023, this category included 4 projects. The largest project, with a total of 1 960 uses of other fish, other amphibians and *Rana sp*., focused on the impact of predation by different fish species on eggs and larvae of three amphibian species. The second largest project, with 1,416 *Anguilla anguilla* uses, focused on the development of methods to characterize the sanitary quality of glass eels so that they can be temporarily housed and vaccinated against anguillid herpes virus 1. In addition, 211 *Discoglossus pictus* were used for research on the impact of the pesticide 2,4-D on amphibian health, specifically examining its effect on the progression of the fungal disease chytridiomycosis in painted frogs. Additionally, 154 other fish (13 *Anguilla anguilla* and 141 *Alosa fallax*) were used to evaluate fish migration through acoustic telemetry and Data Storage Tags (DSTs), also assessing the impact of hydraulic structures on fish migration to provide recommendations for water management authorities.

After a sharp increase in 2022 in the 'Higher education' and 'Training for the acquisition, maintenance or improvement of vocational skills' categories, we see these categories remaining broadly stable in 2023 (+83 and -325 uses respectively, i.e. an increase of 7.51% and a decrease of 6.56%).

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

Overall, we see that in 2023 the proportions of mild use (55.78%) and moderate use (28.49%) are almost the same as in 2022. However, there is a decrease in the proportion of non-recovery uses (from 3.63% in 2022 to 2.25% in 2023) and an increase in the proportion of severe uses (from 11.6% in 2022 to 13.48% in 2023).

The increase in the severe use category is notable. In absolute numbers, this represents an increase of 7 204 severe procedures. This means that the downward trend in the severe category observed in recent years has come to an end in 2023. The increase is mainly due to a rise in the reported severity of applied research on human nervous and mental disorders (+4 988 severe uses) (i.e. epilepsy research), preservation of species (+2 351 severe uses), basic research on the musculoskeletal system (+2 274 severe uses) (i.e. osteoporosis research), and basic research on the nervous system (+2 100 severe uses). In addition, in 2023, as in 2022, relatively high numbers of severe procedures were performed in basic research on the immune system and in oncology.

The decrease in the proportion of non-recovery uses represents an absolute decrease of 6 156 uses in this category. This can be explained by a substantial decrease in non-recovery procedures in basic research on the nervous system (-3 297 non-recovery uses), followed by a decrease in non-recovery procedures in basic research on the immune system (-1 584 non-recovery uses) and a decrease in non-recovery procedures in oncology (-968 non-recovery uses).

The largest number of non-recovery procedures was, as in 2022, in the field of basic research on the nervous system, with 3 512 non-recovery procedures reported in 2023. There were no major outliers in the other categories.

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

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

- In the Brussels-Capital Region and the Flanders Region, continuation of the RE-Place project (database pooling expertise on alternative methods to animal testing) and funding of several specific 3R research projects.

- In the Brussels-Capital Region and the Walloon Region, continued funding of the project that wants to ensure the pooling of equipment and skills (related to the implementation of alternative methods to animal experimentation) of the entire French-speaking scientific community in Belgium. This project should lead to a reduction in the number of animals used over the next few years but at this stage it is too early to see any impact.

- In the Brussels-Capital Region, continued funding for the creation of a Brussels Platform for 3R Alternatives (IC-3Rs). This platform will provide the Brussels-Capital Region with a competent centre for the promotion of animal welfare and the application of the 3Rs in order to have a significant impact, ranging from the increased adoption of innovative techniques and 3R alternatives to the development of policy and regulatory measures.

- In the Flemish Region, monitoring of the action plan developed in collaboration with researchers and organisations to reduce the number of animal procedures in the region and beyond.

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

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

As regards the use of birds, the category other birds accounts for 12.04% in 2023. This category consists mainly of *Parus major* (1,213 uses, or 29.05%), *Numididae* (960 uses, or 22.99%), *Cyanistes caeruleus* (656 uses, or 15.71%) and *Serinus canaria* (619 uses, or 14.82%). These species are mainly used in the categories of animal diseases and disorders (translational and applied research) and ethology, animal behaviour and animal biology (basic research).

Regarding the use of amphibians, the use of other amphibians accounts for 49.77% in 2023. This category consists of the use of *Pelobates fuscus* (800 uses, or 74.70%), *Discoglossus pictus* (211 uses, or 19,70%) and *Lithobates catesbeianus* (60 uses, or 5.60%). These species are used for research relating to the preservation of species and the protection of the natural environment in the interest of human or animal health or welfare.

(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 2023, 61 621 animals, of which 60 726 rabbits, were used for routine production in the category monoclonal and polyclonal antibodies (excluding ascites). This huge increase (versus 169 animals in 2022) is due to misreporting in 2021 and 2022, when rabbits were reported in the category blood based products instead of monoclonal and polyclonal antibodies (excluding ascites). Prior to 2021, no distinction was made between these two categories and animals used for the production of monoclonal or polyclonal antibodies (excluding the method of ascites) had to be reported under the Blood based products category.

Alternative methods are always considered before animals are used for the generation of monoclonal antibodies. The phage display method is often used and tested, but sometimes synthetic monoclonal antibodies are found to be slightly different from animal antibodies.

Animals (mainly rabbits) 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 also note that these mixtures never fully reproduce the performance of the in vivo polyclonal antibody, especially for complex antigens (e.g. cells).

In 2023, 1,305 mice and 38 guinea pigs were used for quality control in the category batch safety testing for abnormal toxicity tests. However, these tests are no longer performed in Belgium. The last abnormal toxicity tests were performed at the end of 2023.

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

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

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

Bulgaria

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

The total number of animals, used for 2023, compared to 2022 is increased. Nevertheless that our country is trying to implement the 3Rs and on the other hand, due to the fact, that some of the sessions in the universities were performed online and/or some of the tests were filmed, during the Covid crisis, most universities had suspended in-person training and lectures for students. Given that permits are valid for 5 years, the actual use of animals started in 2023.

The increased number of animals used is clearly visible for 2023 for the reason described, but in reality, these animals were used for a shorter period of time.
The used animals are only animals born in the EU at register breeder. The number of used rana, other amphibians and mice are reduced, the number of used dogs, rats and domestic-fowl are increased.

(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 2023 animals, compared to 2022 is increased. During the Covid crisis, most universities had suspended in-person training and lectures for students. Given that permits are valid for 5 years, the actual use of animals started in 2023.
In some cases, depending on the project type, the Ethic Commission made recommendations for reduction of the used animals. The project authorization is not given of projects, who do not consider with the recommendation. It was asked, some of the tests to be filmed. On the other hand, this is due to the online sessions in the universities. There are increasingly strict criteria when issuing permits and determining the number of animals allowed for use, and the members of the ethics committee monitor each and every animal used.

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

The procedures with non-recovery severity are reduced, but the moderate and mild severity are increased.

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

The number of re-used animals is increased. This is due to the fact, that most of the tests are with mild and moderate severity. 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:

There are no significant animals, used in category – "other"

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

There are no such animals used.

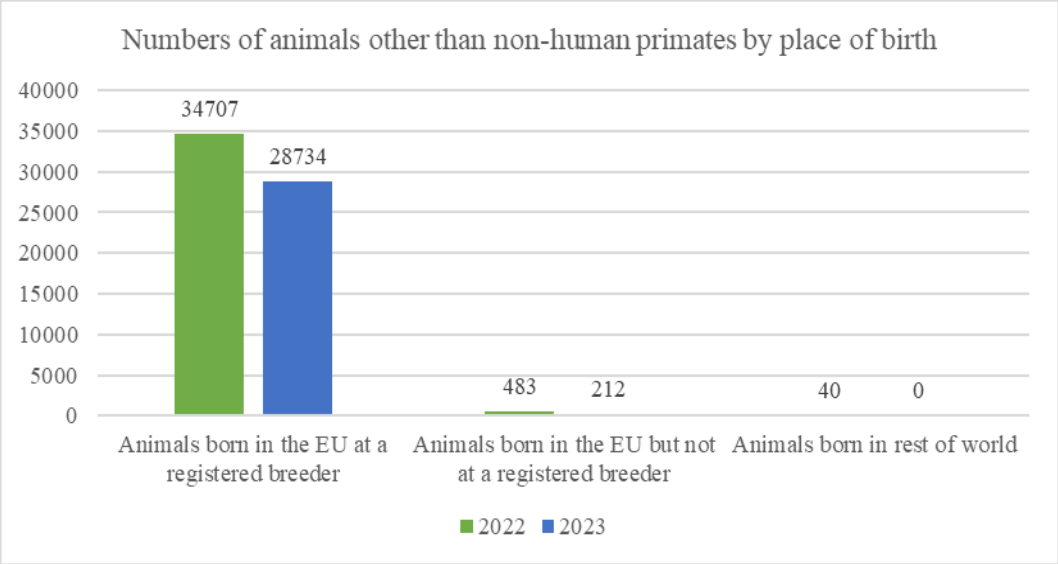
(g) Details on cases where the 'severe' classification is exceeded, whether pre-authorised or not:

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:**

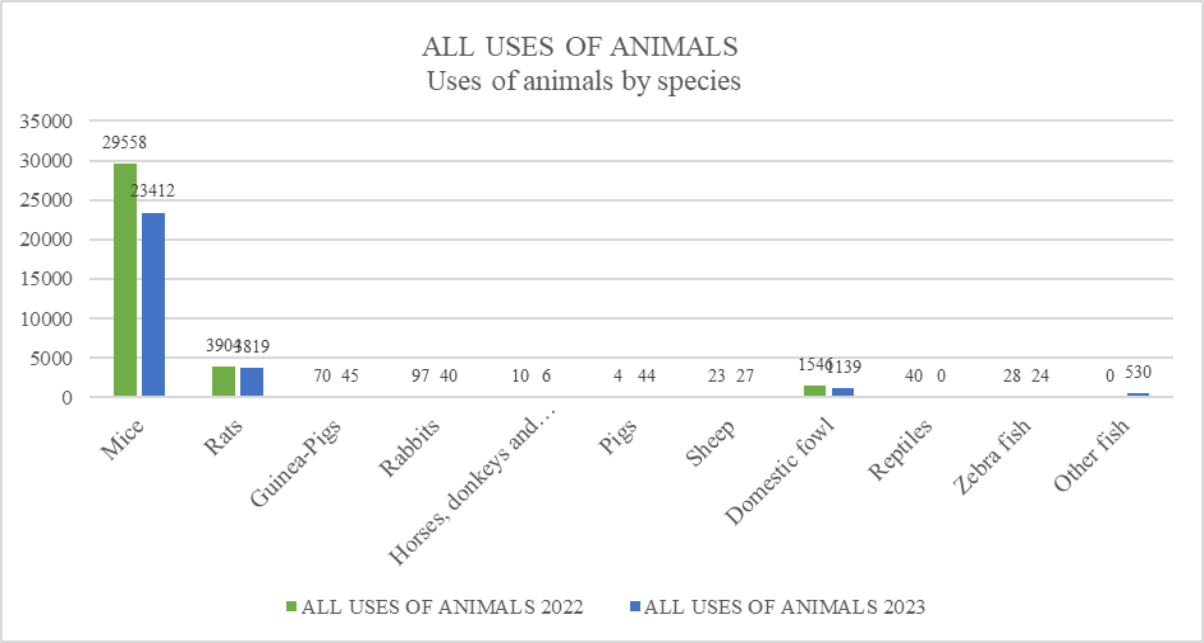
Croatia

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



Main changes observed in relation to the origin of animals between 2022 and 2023 are:

- Animals born in the EU at a registered breeder decreased by 5,973,
- Animals born in the EU but not at a registered breeder decreased by 271,
- Unlike 2022, no animals born in the rest of the world were introduced in 2023.



The main changes observed in relation to the use of animals per species between 2022 and 2023 were:

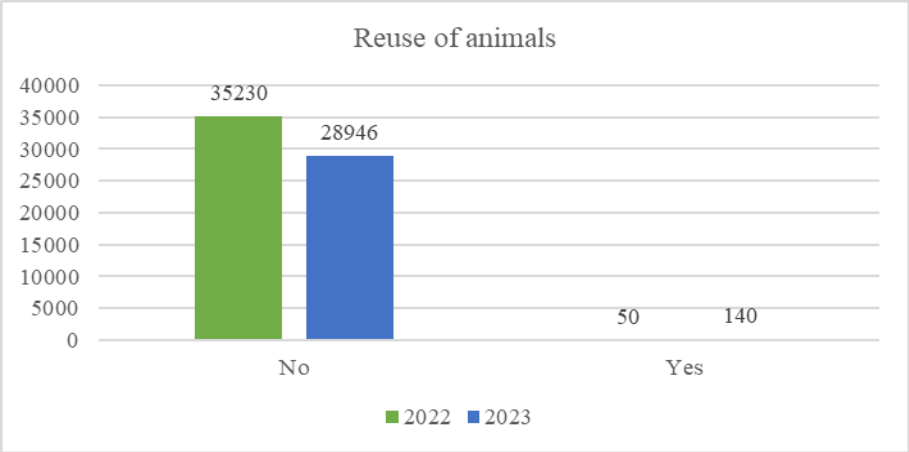
- decrease in the use of mice (-6 146), rats (-85), guinea pigs (-25), rabbits (-57), horses, donkeys and cross-breeds (-4), domestic fowl (-407) and zebra fish (-4),
- increase in the use of pigs (+40), sheep (+4) and other fish (+530),
- none reptiles were used in 2023 compared to none in 2022.

When categorize the total use of animals at cold-blooded vertebrates and warm-blooded vertebrates, the following changes were observed between 2022 and 2023:

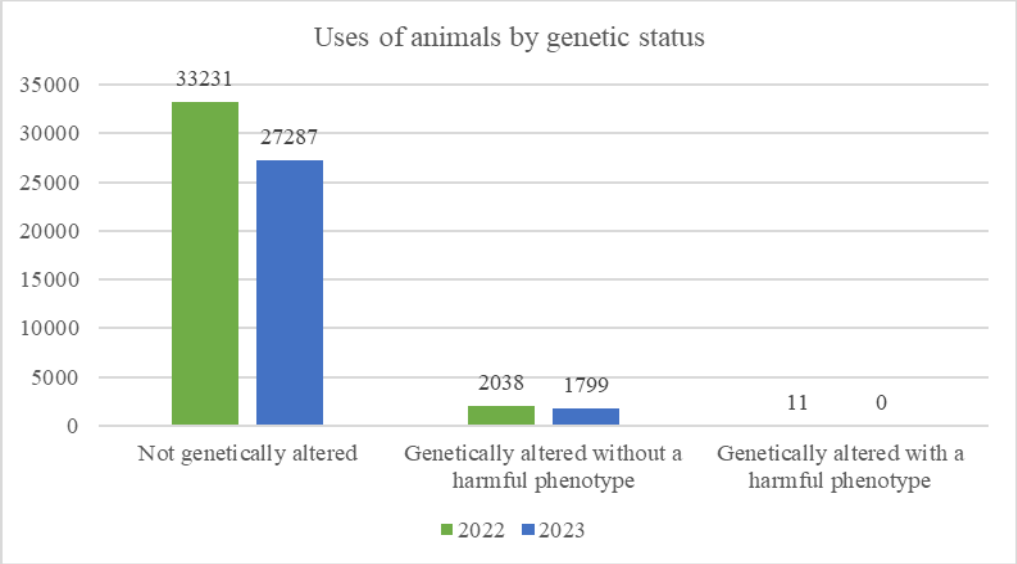
- an increase of 486 cold-blooded vertebrates (fishes),
- warm-blooded vertebrates saw a decrease by 6 680 (birds and mammals).

Regarding the categorisation of animal species, mammals were the most common species used for scientific purposes in 2022, but saw an significant decrease; 6 273 fewer mammals were used.

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



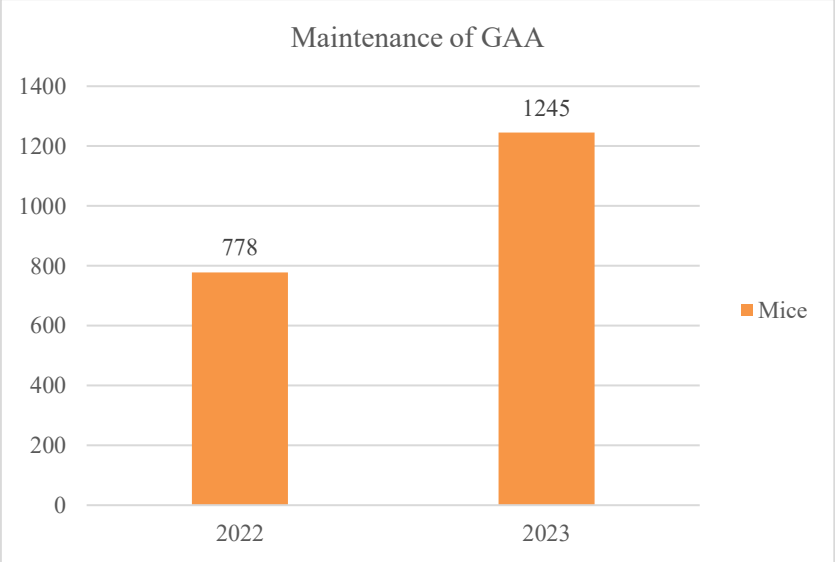
In 2023, a decrease of 6 284 animals that were not reused in scientific purposes was observed, but also an increase of 90 animals that were reused for the aforementioned purposes.



Regarding difference in the genetic status of animals between 2022 and 2023, the following was observed:

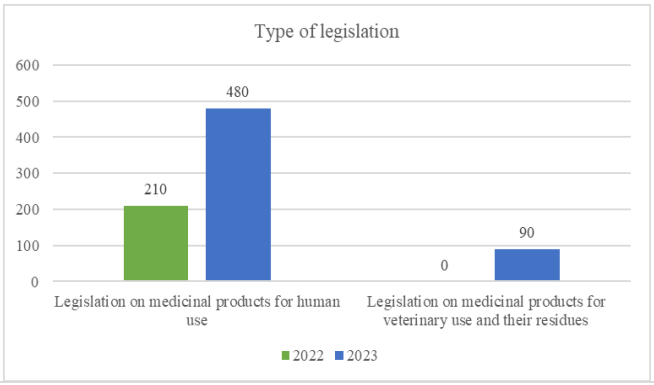
- No of not genetically altered animals significantly decreased by 5 944 animals,
- No of genetically altered animals without a harmful phenotype decreased by 239,
- None of the genetically altered animals with a harmful phenotype were used in 2023.

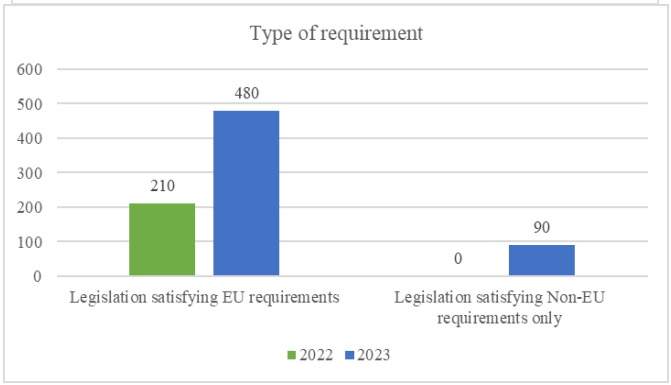
When changes were summed up overall decrease of 6 194 in the difference in genetic status in animals between 2022 and 2023 occured.



467 more mice were used in 2023 to maintain the GAA. Also, all 1 245 mice were used for the first time and all were genetically altered without a harmful phenotype.

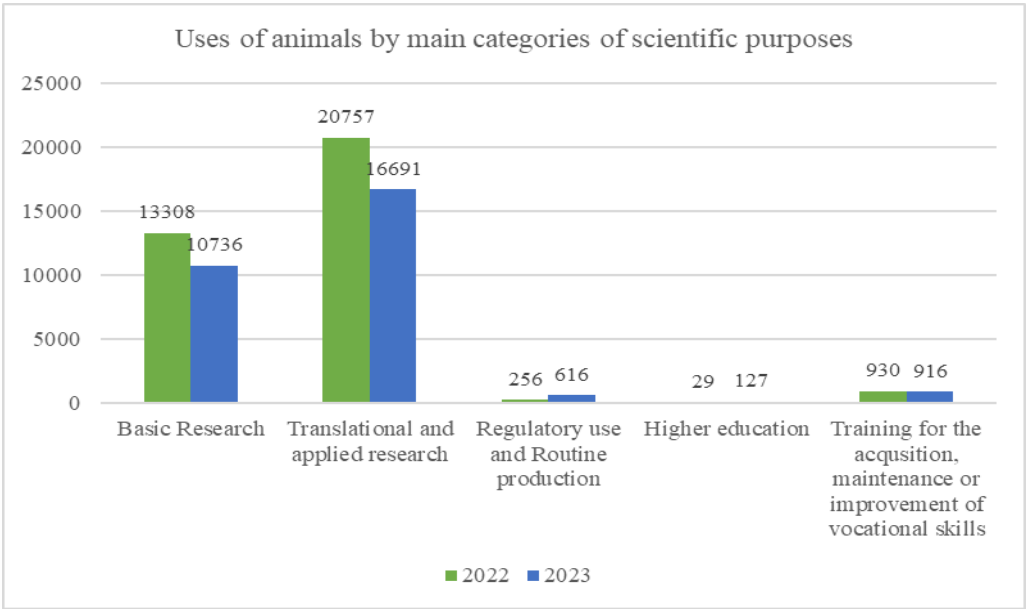
Severity of the procedures was “mild (up to and including)”.





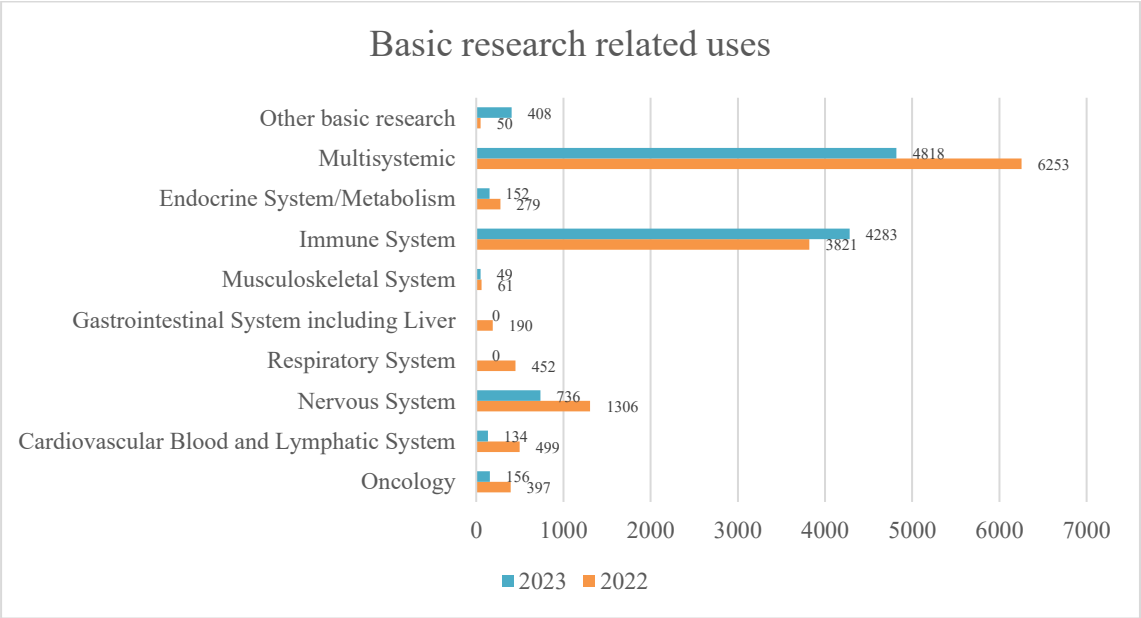
Between 2022 and 2023 there was an increase when it comes to legislative actions related to medicinal product for human use (by 270) and medicinal products for veterinary use and their residues (by 90) which meet EU requirements.

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



- 2 572 fewer animals were used for Basic Research in 2023,
- 4 066 fewer animals were used for Translational and Applied Research in 2023,
- 360 more animals were used for *Regulatory Use and Routine Production* in 2023,
- 98 more animals were used for *Higher Education* in 2023,

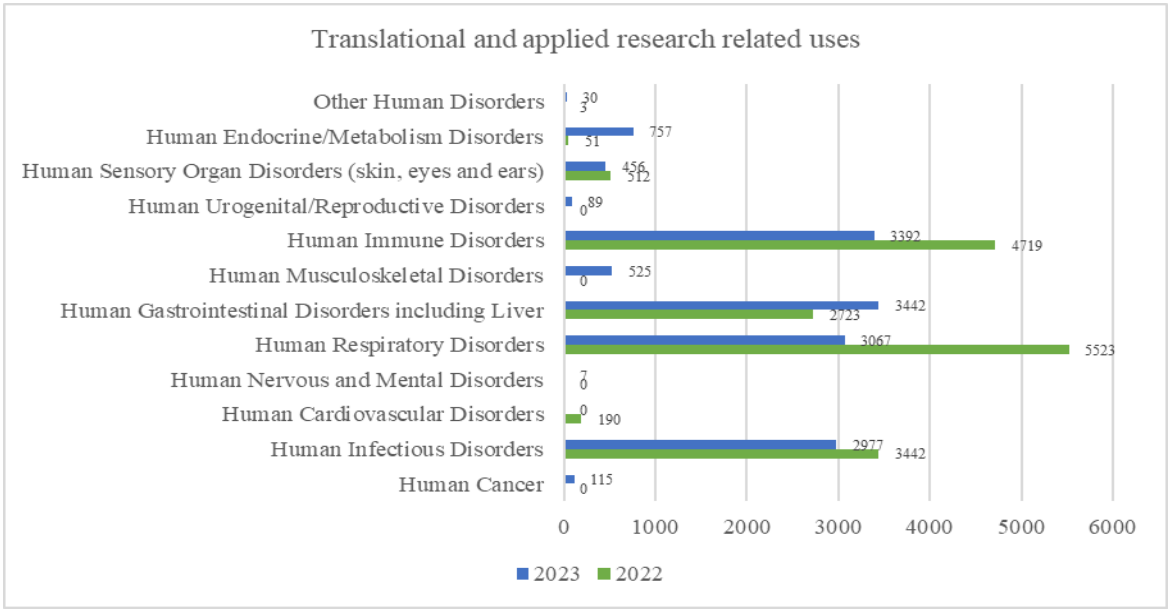
- 14 fewer animals were used for *Training for the Acquisition, Maintenance, or Improvement of Vocational Skills* in 2023.



A significant decrease was recorded in the following purposes:

- *Nervous System*: 570 fewer animals were used in 2023,
- *Multisystemic purposes*: 1 435 fewer animals were used in 2023.

Only significant increase was recorded in *Immune System:* 462 more animals were used in 2023.



A significant increase was recorded in the following purposes:

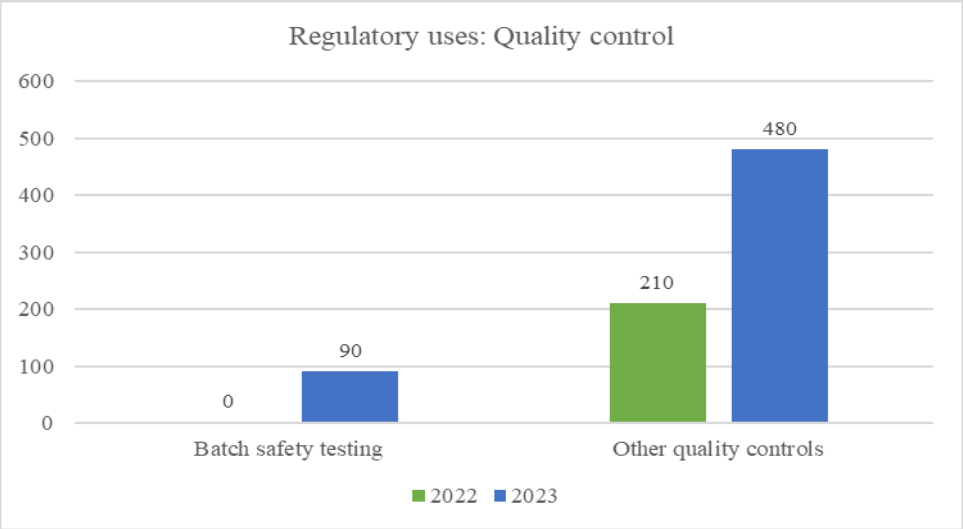
- *Human Gastrointestinal Disorders including Liver:* 719 more animals were used in 2023,

- *Human Endocrine/Metabolism Disorders:* 706 more animals were used in 2023.

Significant decrease was recorded in the following purposes:

- *Human Respiratory Disorders:* 2 456 fewer animals were used in 2023,
- *Human Immune Disorders:* 1 327 fewer animals were used in 2023,
- *Animal Diseases and Disorders:* 2 098 fewer animals were used in 2023.

In 2023, 525 animals were introduced for use in studies on *Human Musculoskeletal Disorders*.



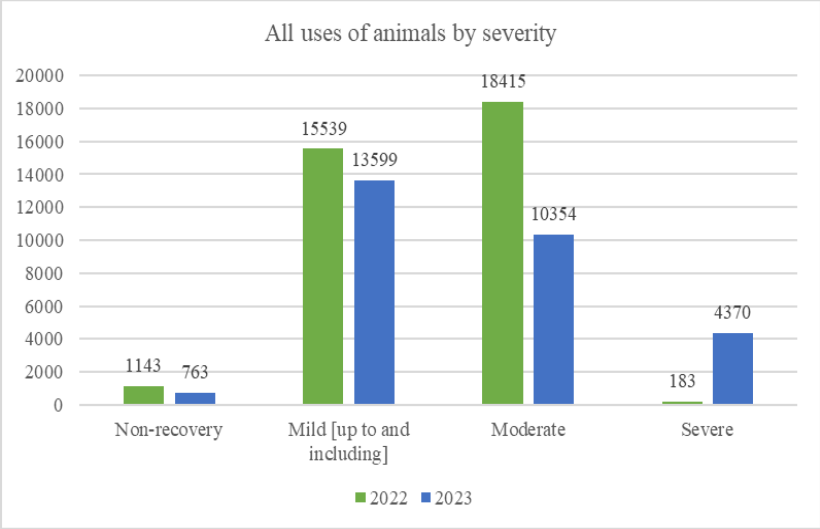
Batch safety testing:

- 90 animals were introduced for *Batch safety testing* in 2023.

Other quality controls:

- an increase of 270 animals used for *Other quality control* in 2023.

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



Non-recovery:

- In 2023, this number decreased by 380 animals

Mild [up to and including]:

- In 2023, this number decreased by 1,940 animals

Moderate:

- In 2023, this number decreased by 8,061 animals

Severe:

- In 2023, this number increased by 4,187 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", 2 animals were used for the purpose of aging, 60 animals were used for the purpose of development of new enteral feed formulations intended for complete or partial diet of patients with eating disorders, 330 animals were used for Eco-Evo-Devo and 16 animals were used for testing of new radio protectors.

In category "Other quality controls" in accordance with regulations on medicinal products for human use, 330 animals were used for the purpose of potency testing of animal blood derivates for human use and 150 animals were used for stability testing and method validation.

(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 2023, 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:

Compared to 2022, both the number of animals used in 2023 and the number of the projects (29 projects in 2023 vs 23 projects in 2022), were slightly increased.

Also, the numbers for severe category were increased significantly (from 10 to 48) because of a new project that was licensed for this category.

(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:

There were no significant changes in actual severities**.**

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

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

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

Not applicable for 2023.

(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 2023.

(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 2023.

Czechia

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

In the Czech Republic the statistical data has been collected since 1993. The trend of decreasing numbers of animals used for scientific purposes continues in 2023, where we can see a lower number compared to previous years. As was the case in the previous year, the number of animals used for scientific purposes in the category "Other legislation" decreased. There was also a decrease in the number of mice used for scientific purposes in 2023. In 2023, there was a slight increase in animals used for scientific purposes in the category "Other fish".

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

From the data obtained for 2023, there is a significant decrease in the number of animals used for scientific purposes in the category "Legislation on medicinal products for human use", as is the case for the category "Medical devices legislation".

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

There is decreasing of moderate uses in 2023. Opposite to this there is increase of mild uses. After continual decreases of non-recovery uses is in 2023 also increase in this category of severity.

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

We are constantly striving to reduce the number of animals used for scientific purposes, in particular paying attention to replacing animals with lower forms of animals. Czech Republic is trying to implement the 3R and consult it with National Committee for the Protection of Animals Used for Scientific Purposes and other professionals, therefore more impacts in subsequent years are expecting.

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

The category "Other birds" accounted for 12.13 % of all birds reported.
Especially: *Phasianus colchicus, Hirundo rustica, Cinclus cinclus, Glaucidium passerinum, Lanius collurio, Parus major, Passer montanus, Erithacus rubecula, Turdus merula, Numida meleagris f. domestica, Cairina moschata f. domestica, Melopsittacus undulatus, Anser anser domesticus, Anas platyrhynchos domesticus, Ficedula albicollis, Cyanistes caeruleus, Acrocephalus palustris, Acrocephalus scirpaceus, Acrocephalus schoenobaenus, Aegithalos caudatus, Anthus trivialis, Carduelis cannabina, Carduelis chloris, Carduelis carduelis, Certhia familiaris, Coccothraustes coccothraustes, Emberiza citronella, Emberiza schoeniclus, Fringilla coelebs, Hippolais icterina, Locustella fluviatilis, Locustella luscinioides, Locustella naevia, Motacilla alba, Poecile palustris,*

Phylloscopus collybita, Phylloscopus sibilatrix, Prunella modularis, Serinus serinus, Sitta europaea, Sturnus vulgaris, Sylvia atricapilla, Sylvia borin, Sylvia communis, Sylvia curruca, Serinus canaria, Turdus philomelos, Turdus viscivorus, Periparus ater, Picus canus, Dendrocopos major, Columba palumbus, Streptopelia decaocto, Cuculus canorus, Scolopax rusticola, Buteo buteo, Columba livia f. domestica and Anas platyrhynchos.

In category "Other Legislation" are included mainly experiments for the purposes of waste legislation and toxicity of waste. In total, this category accounted for 44.63 % of the observed types of categories of uses by type of legislation.

Category "Other product types" was used mainly for experiments that were for purposes of acquisition of live attenuated lines of selected species of chicken coccidian for vaccine production and experiments that were for production of antigen of Rabbit haemorrhagic disease. In total, this category accounted for 97.46 % of the types of categories of routine production uses monitored.

(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 uses" were 540 mouses use for production of "Monoclonal antibodies by mouse ascites method" which is 2.30 % of the total number within the category "Routine production uses". In this category were 55 rabbits use for production of "Monoclonal and polyclonal antibodies (excluding ascites method)" which is 0.23 % of the total number within the category "Routine production uses". The remaining 97.46 % is classified as "Other product types" which is described above.

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:

- **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

Classification "severe" was not exceeded in 2023.

Denmark

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

The number of laboratory animals in 2023 is similar to the number in 2022. The most frequently used animals remain mice, rats, pigs and fish.

Denmark expects the increase in the number of dogs used to be permanent, as one company has increased its capacity for carrying out trials on dogs.

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

- The reason for the decrease in the number of rabbits used is that a major contract research laboratory no longer carries out GMP batch release testing.

- The reason for the increase in the number of dogs used is that one company has increased its capacity for carrying out trials on dogs.

- The reason for the decrease in the number of pigs used is the completion of a major research project on the transport of pigs.

- The reason for the increase in the number of sheep used is two new research projects dealing with the development of new implants and vascular prostheses for humans, respectively.

- The reason for the increase in the number of cattle used is a major research project to develop a screening tool for the early onset of certain diseases, such as subclinical ketosis. This information has been obtained from a single blood sample taken from each animal.

- The reason for the increase in the number of hens used is two research projects which concern the mapping of sternum fractures, and the EU SPRINT project, which examines sustainable plant protection products and their effects on ecosystems, plants, animals and humans.

- Among other mammals, there is a decrease in the number of hedgehogs used. This is due to the completion of a major research project on the potential danger to hedgehogs posed by robot lawn mowers.

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

In Denmark, very few animals are subject to the highest level of severity.

The proportion of zebrafish subject to the highest level of severity rose from 0.27% in 2022 to 4.62% in 2023. All zebrafish subject to the highest level of severity were included in experiments with acute toxicity tests carried out in accordance with OECD guidelines. (The

Danish 3R-Center is in contact with the research groups concerned to see if the experiments could be concluded before the fish experience the highest level of severity without jeopardising the experimental results (see point (d) for further details)).

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

Considerable efforts are being made in Denmark by both the Danish 3R-Center and the Danish National Committee for the Protection of Animals used for Scientific Purposes. For instance, they organise an annual international 3R symposium for researchers in the field, an annual meeting of the country's animal welfare bodies, cooperation with 3R organisations internationally, the distribution of research funding for 3R research, and extensive communication activities on two websites (3rcenter.dk and natud.dk).

In 2023, as part of an international cooperation project the Danish 3R-Center developed a guide to assessing the welfare of laboratory animals. The guide can be used when assessing whether animal welfare meets the requirements that an organisation or company may have in place. It describes the concrete issues relating to animal welfare that need to be taken into account when establishing external cooperation (link).

Meetings were held in 2023 and 2024 with research groups using animals in the highest severity category ('significant') to discuss humane end-points and ways to reduce stress. These are research groups working on vaccine and infection trials on fish and acute toxicity tests on fish, as well as groups working on the EAE model. This has resulted in an improved score matrix for the EAE model, while the work on fish subject to significant severity is still ongoing.

The National Committee for the Protection of Animals used for Scientific Purposes gave a presentation on its cooperation with animal welfare bodies to the Norwegian national committee for the protection of laboratory animals.

The Danish 3R-Center also met representatives from Australia and New Zealand who were interested in learning more about its organisation, as there are plans to establish a 3R centre for Australia and New Zealand.

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

There were 109 laboratory animals reported in the category 'other amphibians', the vast majority of which (94%) are axolotls. They were used to gain an understanding of the processes that allow the axolotl to regenerate tissue.

There were 420 laboratory animals reported in the category 'other birds'. The most frequently used species in this category is the zebra finch (21%). The remaining 79% comprises 21 other species of bird, including the Eurasian blackcap (15%) and the willow warbler (12%).

Zebra finches are used, inter alia, to develop a new model organism to identify more effective physiological mechanisms for oxygen transport in tissue and to establish how the ear of this bird species has evolved and how this has affected its directional hearing.

The remaining species of bird are used under the same authorisation, under which the purpose is to monitor zoonotic diseases and the spread of diseases in 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:

No cases of animals being used in procedures where there is a recognised alternative have been recorded in Denmark.

(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:** A
- **Details of the use:**
- **Reasons why the 'severe' classification**

Denmark has not significantly exceeded the classification in 2023.

Estonia

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

Number of animals used has been decreasing in the previous years. The decrease has slowed down, but number of uses is somewhat smaller in 2023 (3306 animals) compared to 2022 (3324 animals).

Warm-blooded vertebrates (mammals and birds) have been the most used animals by category each year with mammals being most used. Use of birds is the lowest it has been since 2020. Small decrease can be seen in the use of cold-blooded vertebrates (fish) compared to 2022.

There are some changes in the number of animals used by species. Use of mice has been in a steady increase; use of cattle has also increased compared to previous years. For the first time, sheep have been used in 2023. Use of rats and other birds has decreased significantly compared to 2022.

Additional changes include reduced use of domestic fowl and rabbits in 2023. Use of pigs has stayed the same during 2022 and 2023. Also, use of "other" animals has decreased in 2023, and this accounts for 4.48% of all uses (significant drop compared to 16.18% in 2022). No "other" rodents were used in 2023. "Other" birds in 2023 include great tits (*Parus major*), "other" fish include Eurasian perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*).

	2022		2023	
Species	**Number**	**%**	**Number**	**%**
Cattle	243	7.31%	493	14.91%
Domestic fowl	139	4.18%	116	3.51%
Mice	1838	55.29%	2146	64.91%
Other birds	376	11.31%	28	0.85%
Other fish	161	4.84%	120	3.63%
Other rodents	1	0.03%		0.00%
Pigs	4	0.12%	4	0.12%
Rabbits	51	1.53%	42	1.27%
Rats	511	15.37%	245	7.41%
Sheep		0.00%	112	3.39%
Grand Total	**3324**	**100.00%**	**3306**	**100.00%**

There has been a steady decrease in the use of genetically altered animals during the previous four years and in 2023, the uses showed an increase. In 2022, 9.63% of all animals used were genetically altered, in 2023 it was 13.91%.
Of all genetically altered animals used, majority are mice (80.4% in 2023) and the rest are rats.

All animals used in 2022 and 2023 were born in the EU. The number and proportion of animals born in the EU at an authorised breeder have remained similar through 2022 (2400

animals, 72.2%) and 2023 (2 433 animals, 73.59%). No animals were reused in 2022 and 2023.

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

Use of animals for basic research has been in a decline for the past years (~70% in 2021, ~63% in 2022 and ~43% in 2023), while use of animals for translational and applied research has been increasing (~12% in 2021, ~27% in 2022 and ~51% in 2023). No significant change in other fields of research compared to 2022.
Changes compared to previous year are mostly due to research fields that scientists choose to pursue, the funding they get, and this reflects in the types of projects authorised.

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

Proportion and number of uses in mild procedures has been increasing while uses in moderate and non-recovery procedures has been decreasing slightly during the past years. Uses resulting in severe severity have increased in 2023. While in 2022 several animals that were used in experiments with an expected severity of severe were reported as having experienced mostly moderate severity, in 2023 there were more animals with the same expected and actual severity (severe).

	2021		2022		2023	
	Number	**%**	**Number**	**%**	**Number**	**%**
Mild [up to and including]	2 052	52.80%	2 116	63.66%	2 190	66.24%
Moderate	1 623	41.77%	1 080	32.49%	918	27.77%
Non-recovery	117	3.01%	123	3.70%	72	2.18%
Severe	94	2.42%	5	0.15%	126	3.81%
Grand Total	**3 886**	**100.00%**	**3 324**	**100.00%**	**3 306**	**100.00%**

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

In 2023 a new project evaluation committee was formed. Also, projects are now authorised by the same competent authority in charge of inspections, which will improve the promotion of replacement, reduction and refinement (the 3Rs) during project evaluation, authorisation and inspections. During the evaluation of project proposals all justifications are reviewed to ensure the 3Rs are appropriately considered and applied. Animal Welfare Bodies also make sure that the 3Rs are implemented. Laboratory Animal Science courses take place every year, where this is covered.

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

N/A

(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:

146 animals (104 domestic fowl, 42 rabbits) were used for the purpose of routine production of monoclonal and polyclonal antibodies, excluding ascites method in 2023. In the case of 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, when necessary, live animals are used to obtain biologically active and/or highly specific antibodies that could be used for both therapeutic and *in vitro* diagnostic purposes.

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

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

No such cases in 2023.

Finland

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

The overall picture changed little from the previous years, although the total number of uses was decreased by approximately 20 000. The number of procedures totalled 73 280 animals (compared to 93 441 in 2022). The decrease in the number of uses was mainly seen in mice: -6 917 procedures for the creation of new genetically altered animal lines and -7 115 procedures in other uses. The use in the creation of new genetically altered lines was now at a conventional level after a period of a couple of years with more active working. The reduction of other uses in mice was due to decreased use by some actors without clear reasons. The rat procedures were also slightly decreased.

The number of procedures with cats remained at the previous years' levels, whereas the dog procedures were decreased. These species were used mainly in the research projects in which disease genes or metabolic parameters were studied (pets, 231 cats and 527 dogs). In these projects, single blood samples were taken from pet animals. Moreover, pets (1 cat and 158 dogs) participated in patient studies. The dogs bred and used in the laboratory were involved in 80 procedures.

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

The changes of procedures in different research areas can mostly be considered as a normal annual variation in animal research. The decreasing tendency was seen in basic and translational research, where mice are the most used animals. The research areas with high animal numbers were Nervous system (basic research) and Human nervous and mental disorders (translational research). The use of animals for these purposes has shown a decreasing trend in recent years: Nervous system from year 2020 to 2023: 9 087 -> 5 364, and Human nervous and mental disorders 19 968 -> 12 596.

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

Severe procedures (2 196) were decreased as compared to the years 2020 (4 763), 2021 (4 687) and 2022 (3 401). The main purposes for the use of animals (mice and rats) in severe procedures were Nervous system (basic research) and Human nervous and mental disorders (translational research): these purposes included 1 702 severe procedures, which is 77.5 % of all severe procedures. Hence, the decrease of severe procedures is presumably due to the decrease of animal use in these purposes, see above.

The reduction in severe procedures may also be a result of the effective use of 3Rs and more careful assessment of the actual severity of procedures for each animal. Moreover, testing of new medicinal candidates in animal models as a service depends on the needs of customers. This kind of animal use may vary greatly in different years.

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

FIN3R – the Finnish 3R Centre https://fin3r.fi/en organises education and training on 3R issues for researchers. The year 2023 included the FIN3R Annual Symposium (Improving the quality of biomedical research through the 3Rs principle), and six webinars/seminars, which are all available as recordings. The centre also actively disseminates information about the events organised by other 3R centres. Sharing of knowledge about 3R possibilities is important to improve the quality of research and the welfare of animals.

TOKES, the Finnish national committee (Council on the protection of animals used for scientific or educational purposes), has a group for 3R activities. The group works with FIN3R, and it follows 3R activities in Finland. The work has just recently started.

The Project Authorisation Board produces and publishes guidelines with current references, including issues with project application, experimental techniques, surgical operation, anaesthesia and analgesia: https://avi.fi/en/services/individuals/licences-notices-and-applications/animals/laboratory-animals.

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

Other rodents used were bank voles. Other carnivores were Lake Saimaa ringed seal and foxes. Other birds were several wild bird species. Other fish were cichlids, sand gobies and three-spined sticklebacks.

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

In routine production, polyclonal antibodies were produced in swine, sheep and rabbits for diagnostic purposes, altogether 600 procedures. The necessity to produce antibodies in animals was discussed with the producers during their project license process. The reasons for the use of animals were difficulties in finding non-animal methods with enough reliability and effectiveness to produce antibodies in large volumes.

(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**

Not exceeded.

France

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

The 2023 survey comprises responses from all of the establishments approved for the use of animals for scientific purposes (user establishments). As a result of requests for new establishments, mergers and closures, the number of establishments has grown from 575 in 2022 to 589. Of these 589 establishments, 56 stated that they had no uses to report for 2023.

In 2022 the Commission updated its guidance on genetically altered animals, thereby extending the scope of the survey (inclusion of data on farm animals genotyped by techniques not allowing identification but not used in an experimental procedure). As a result of this change in method for the years 2022 and 2023, the number of animals declared in section 3 (maintenance of colonies and creation of lines) of the ALURES database is on the rise. These animals were previously reported in the five-year statistics. This change has led to an increase in the number of animals declared in section 3 from 278 199 in 2022 to **539 351** in 2023.

The number of animals used for the first time (section 1 of the ALURES database) went from 1,829,827 in 2022 to **1 491 162** in 2023, meaning a decrease of 18%. The number of animal uses (section 2 of the ALURES database) went from 1,849,859 in 2022 to **1 507 403**, i.e. it decreased by 18% compared to 2022. The number of animals declared in section 3 includes both animals used to create new lines, the number of which animals fell from 52,764 in 2022 to **47 573** in 2023 (i.e. a drop of 10%), and animals used for colony maintenance. The increase from 225,435 in 2022 to **491 778** in 2023 is due to the change in method (118%).

The total number of animal uses (sum of sections 1 and 3) declared in 2023 is **2 046 754**, compared to 2 128 058 in 2022, i.e. a decrease of 3.8%.

Species used

The 2023 figures confirm the dominance of the mouse model in experimental procedures (**68%** of uses). Rabbits (**8.7%**) and rats (**7%**) remain the two next most used species. Fish account for **8%** of uses, all species combined.
The number of uses of cats stands at **951** compared to 1 127 in 2022, or a 16% decrease. These uses are largely for regulatory tolerance tests for veterinary drugs (48%) or studies regarding nutrition or veterinary medicine (39%). Of these uses, **69%** are classified as 'mild'.

Uses of dogs increased by 3.7%, with **4 107** uses (3 961 uses in 2022). The proportion of reuses is 36%. Of these uses, 71% are classified as mild. The tests carried out on dogs mainly concern regulatory toxicology in human or veterinary medicine (58%).

Uses of primates decreased from 4 147 in 2022 to **3 459** in 2023, a reduction of 17%. Of these uses, 46% concern regulatory testing in human medicine and 21% concern the production of biological fluids (mainly blood products), compared to 30% in 2022. These

products are used by laboratories in France, but two thirds of them are also exported to other European countries and third countries.

The number of primates used for the first time was **2 372** in 2023 compared to 3 021 in 2022. This decrease of 21% could be explained by a decrease in needs after a significant increase in activity linked to the COVID-19 pandemic. However, it cannot be excluded that the decrease is a consequence of the supply difficulties experienced in recent years or of transfers of these activities to other countries with lower animal welfare standards than in Europe. It will be necessary to remain vigilant in the coming years.

The number of primates of second generation or higher (F2) or from self-sustaining colonies (SSC) is 1,713. The 658 first-generation primates (F1) are cynomolgus monkeys (*Macaca fascicularis*) only. These data show that the transition to the use of F2 generation or SSC animals has been achieved for the other primate species. Owing to supply difficulties, sufficient time should be allowed to carry out the transition for cynomolgus monkeys.

A wild-caught primate (generation F0) was reported. This animal, caught in 1996, was part of a project to investigate prion transmission risks in transfusions. It left the study project after 24 years of incubation.

The proportion of primates being reused was **31%**, compared to 27% in 2022.
Salmonids (27 308 uses) and moronidae such as sea bass (15 162 uses) account for 26% of uses of fish (compared to 44% in 2022). This decrease represents a return to the figures recorded in 2021. Research projects involving these fish relate, in particular, to the study of environmental changes, such as global warming. Other projects concern the optimisation of livestock by genetic selection or the development of probiotics with a view to pathogen resistance and growth improvement.

The number of uses of birds has grown by 12% compared to 2022. There were 14 317 uses of turkeys (*Meleagris*), of which 13 931 relate to the production of a vaccine strain for this species.

Reuses

All species combined, the number of reuses was **16 241** in 2023 compared to 20 032 in 2022. This corresponds to an overall reuse rate of 0.8%. This rate is higher for large species with longer lifespans such as carnivores, certain livestock, such as bovines, or primates.

The apparent decrease in reuse compared to 2022 could be due to the work carried out to explain the definition of reuse to user establishments.

Genetically altered animals

The proportion of uses of genetically altered animals was **43%**, as compared to 39% in 2022. The vast majority of these animals were mice (**91%**). The proportion of phenotypes

identified as harmful has remained stable in recent years, with **4.2%** in 2023 and 5.1% in 2022.

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

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

- basic research (31%);
- maintenance of colonies of genetically altered animals (24%);
- regulatory uses or production of biological products for therapeutic use (22%);
- translational or applied research (19%).

These four areas account for 96% of uses. As in 2022, taking into account the expanded scope of data collection to include the maintenance of colonies, the proportions for all the other areas have seen a corresponding decrease.

The share of animal uses for basic research decreased from 39% in 2022 to **31%** in 2023. Once again, the field of neuroscience used the most animals, with 183 835 uses, followed by immunology and oncology with 114 014 and 93 709 uses respectively.
Given the expansion of the survey scope, colony maintenance (section 3) accounts for **24%** of uses, compared to 11% in 2022.

Toxicological and regulatory research for the development, production or quality and safety testing of medicines or foodstuffs follows, with **22%** of uses (24% in 2022). In this area, there are 170 363 uses of rabbits 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 growing demand due to proven efficacy.

Translational or applied research decreased from 23% in 2022 to **19%** in 2023. Among human diseases, a large share was attributable to research on cancer (29%) and infections (16%). Uses relating to animal welfare or animal diseases (10%) mainly concern species intended for human consumption, as well as pets, to a more limited extent.

Other scientific fields remain considerably less represented.

- Species conservation remains stable, with 2.3% of uses. Fish studies account for **94%** of cases. To give an example, a project on estuary restocking required the use of 17 246 animals. These projects concern multiple wild species including eel, mullet, meagre, sea bass, flounder, whiting, sole, sturgeon, lamprey, conger, minnow, roach and bullhead.
- The area of education and vocational training remains comparable to previous years at **1.7%** 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 (**87%**). Uses falling under the category 'non-recovery' are stable, comprising **3.2%**.

Uses classified as 'severe' have fallen, with **9.3%** as compared to 12% in 2022 and 14% in 2021. While in 2022 this decrease resulted from the expanded scope of data collection, it is also due to better training of the professionals concerned in the actual assessment of severity (webinars carried out by the Ministry).

(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'). This work is carried out through several lines of action.

The FC3R

A national centre dedicated to the 3Rs principle - the FC3R - was established in 2021. The centre's mission is to fund research on the development of alternative methods, to develop and disseminate training on the application of the 3Rs, to support research projects by providing engineering in strict compliance with the 3Rs, to encourage the deposition of animal lines, to make unpublished data or negative results accessible in order to avoid unnecessary duplication of projects, and to ensure transparent communication on the use of animals for scientific purposes.

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

After three years of operation, the FC3R centre now has a high profile nationally. A website with a large amount of information and resources was launched in June 2022: https://www.fc3r.com/. The decrease in the number of animal uses (section 2) observed in 2023 can be linked to the activity of the FC3R.

Three calls for projects have already been carried out. A total of 253 applications were submitted and 35 projects were funded. A fourth call for projects entitled 'Innovate, Replace, Share' ('innover, remplacer, partager') was closed in 2024. Alongside this, the FC3R is preparing an inventory of the existing alternative methods in France.

The FC3R has set up four working groups on the following topics:

- The 3Rs in education and training
- The circulation and accessibility of 3R information
- Severity assessment and uses classified as severe
- Assessment and success indicators for the 3R policy

These working groups are expected to present their conclusions in the course of 2025.

The National Committee

The French National Committee is composed of two bodies: the National Committee for the Protection of Animals used for Scientific Purposes (CNEA) and the National Committee for Ethics in Animal Research (CNREEA). These bodies proposed several recommendations in 2023 and recently worked together on a recommendation for the training of members of ethics committees. This recommendation is currently being implemented in consultation with the FC3R.

Professional associations

Professional associations for animal research and national networks of ethics committees and animal welfare monitoring structures also have a prominent role in the field. They carry out important activities (annual congresses, national thematic days, lists for exchanging best practices, national and regional seminars) in all areas related to the 'culture of care'. The ministerial authorities collaborate fruitfully with these bodies.

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

Declarations in the category 'other' are always subject to thorough checks in order to minimise their number.

For basic research, no uses in the category 'other' have been declared. For regulatory uses/routine production, the category 'other' accounts for **3.7%** and includes in particular the production of toxoplasm for toxoplasmosis testing kits, the production of a vaccine strain for veterinary use and the production of a protein for therapeutic use. These three projects, which address public health issues that do not fall into the pre-defined categories of the statistical survey, account for 88% of the 67 191 uses classified as 'other' in this category.

As in 2022, the 'other fish' category remains sizeable, accounting for **2.8%** of uses (57 304). They related to numerous environmental studies, including climate change. This category includes diverse species such as sole, eel, sturgeon and three-spined stickleback, to name but the main ones. A project on infectious disease models for farmed fish, aimed at developing health products for aquaculture, required the use of 15 541 animals. Nearly two thirds of these uses concern immunity studies (15 541 uses) or species conservation studies (16 595 uses).

The 'other birds' category accounted for **0.5%** of uses (10 979). This category includes domestic species such as ducks, as well as wild species such as tits, kingfishers and dippers. 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 **35 424** uses, compared to 49 038 in 2022. This 28% decrease results from the approach followed for a number of years, with monitoring of existing authorisations and extensive

dialogue with the user establishments concerned. This helps to support the authorisation holders as they put in place robust transition plans, aiming at as rapid a transition as possible to *in vitro* production while avoiding a transfer of activities to countries with lower animal protection standards than in Europe.

These monoclonal antibodies are used by hospitals and laboratories in France and abroad for biomedical diagnostics. The need to maintain continuity of care provision has led to authorisations being renewed for a limited period. The establishments have all initiated efforts to develop alternative methods but some are still encountering difficulties with analytical quality, quantities, robustness of tests or time needed for validation by the authorities of the countries concerned.

With a view to the withdrawal of the 'rabbit pyrogen test' (RPT) from the European Pharmacopoeia in 2025, a similar approach has been taken with establishments that hold this authorisation. A significant decrease of 28% was observed in 2023, with **5 161** uses compared to 7 145 in 2022. However, two areas of concern remain. The first has to do with vaccines produced in Europe and exported to third countries that maintain the RPT obligation. The second concerns the use of this test for medical devices that are outside the scope of the European Pharmacopoeia.

Work to reduce the number of animals used in skin and eye irritation tests continued in 2023. There is again a significant decrease of 15% compared to 2022 in uses for skin irritation (30% compared to 2021), 11% for skin sensitisation and 10% for eye irritation. Many of these uses are still referred in harmonised standards for the validation of medical devices biocompatibility; changes in these standards will help to further reduce the use of animals.

In response to the Commission's expectations, specific work on the RAT list (Replace Animals Tests) is being undertaken. Initial results show that uses potentially identified as botulinum toxin tests (items: 'batch potency testing' + 'mice' + 'human medicines legislation') are in fact efficiency or quality of production checks linked to other pathogens. Similarly, uses presumed to be leptospirosis tests (items: 'batch potency testing' + 'hamster' + 'veterinary products legislation') in fact include assessments of the effectiveness of a vaccine against leptospirosis using vaccine challenge tests on rodents.

Although there are validated alternative methods in Europe, the hamster test is carried out in response to requests from third country authorities.

(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:**

No authorisations were issued in 2023 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 2023, approximately 1.46 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 16% lower than in the previous year (2022). This continued the downward trend from previous years.

Around 80% of the animals used for testing are rodents, mainly mice and rats. Mice accounted for around 73% of the animals used and rats for around 7%. Some 11% of the animals were fish, around 5% were rabbits and around 1% were birds. These proportions were more or less the same as in the previous year. When compared over the long term, there were no substantial changes in the distribution of animals used either. For example, the figure for mice increased by 0.6% compared to the previous year (around 72% in 2022), continuing the gradual increase since 2019 (around 72% in 2021, around 71% in 2020 and around 65% in 2019). The figure for fish (around 12% in 2021 and 2022) changed by 1% compared to previous years. The use of birds and rabbits also did not significantly change compared to 2022. The proportion of other animals used for testing remained stable at around 3% compared to the previous year (around 3% in 2022 and around 4% in 2021).

Going beyond the requirements of the EU Laboratory Animals Directive, 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. Some 672 000 animals were used for this purpose in 2023. This represents a reduction of around 6% compared to 2022 (around 712 000 animals). The raw data on these animals killed pursuant to Section 4(3) of the Animal Welfare Act will not be sent to the European Commission for the 2022 reporting year. Since the 2021 reporting year, every year Germany has also had to report all animals that were bred for animal testing or for the scientific use of their organs or tissues, but that were not used for such purposes and were instead killed. In 2023, around 1.37 million such killings were reported. This represents a reduction of around 22% compared to 2022 (around 1.77 million animals). Again, the raw data on these animals reported pursuant to the first sentence, point 1a, of Section 1(1) of the Regulation on the reporting of vertebrate animals or cephalopods used for testing purposes or vertebrate animals used for certain other purposes (*VersTierMeldV*) will not be sent to the European Commission for the 2022 reporting year.

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

Genetically modified animals

The number of genetically modified animals used decreased significantly compared to the previous year, with around 738 000 instances in which genetically modified animals were used (around 918 000 in 2022). In addition, the proportion of genetically modified animals out of the total number of animals used also decreased. In 2023 the proportion was around 51%, bringing an end to the upward trend of recent years (around 53% in 2022, around

51% in 2021 and around 48% in 2020). This concerned in particular mice (87%) and fish (12%). In 2023, the number of rats used to produce new genetically modified lines was back to 320. This represents a reduction of 140 animals compared to the previous year. The number of genetically modified domestic chickens (541 animals, down from 990 in 2022) and pigs (667 animals, down from 884 in 2022) has also almost halved. This has broken the continuous upward trend since the 2018 reporting year in the number and proportion of genetically modified domestic chickens and pigs.

It is also particularly noteworthy that the number and proportion of animals bred, subject to authorisation, in order to conserve colonies of genetically modified animals, but subsequently not used in further procedures, more than halved compared to 2022 (55% decrease). Most recently, the figures for these animals had risen compared to previous years (around 190 000 animals in 2022 and around 157 000 in 2021). It is worth noting here that, compared to previous years, fewer zebrafish were reported for this testing purpose (around 7% in 2023, around 12% in 2022 and around 11% in 2021).

Primates

Following 2022's increase in animal testing using primates (2 204 uses in total), 2023 saw a significant reduction, down to a total of 1 676 uses (down by 24%). Around 89% of these were for regulatory purposes or routine production. In 2023, a total of 1,489 primates were reported for the first time, while the remainder (187 animals; around 11%) were animals being reused. Of the animals used for the first time, around 96% came from self-sustaining colonies, of which around 12% came from Europe, around 20% from Africa (39% in 2022) and around 69% from Asia (47% in 2022). The origin and pedigree of the animals changed compared to the previous year, with more animals originating in Asia.

As in 2022, mainly long-tailed macaques were used in 2023 (around 88% of uses in 2023 and 86% in 2022).

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, 2 550 uses of dogs and 544 uses of cats were reported (2 874 and 538 respectively in 2022). Compared to 2022, the number of uses of dogs decreased by around 11%, while the figure for cats remained almost unchanged.

Scientific purposes

Although many scientific questions can be answered nowadays through the use of substitute methods and computer-assisted procedures, it is not yet possible to do so without the use of animals for scientific purposes, including in biomedical research. Specifically, some 59% 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 6% of the animals used were needed to maintain colonies of established genetically modified animals and could not be used in other procedures. Around 4% were needed for

other purposes, such as training or further education, protecting the natural environment or species protection. There were no major changes compared to 2022.

The proportion of testing in the area of basic research increased by 4%, while translational research and regulatory use changed by no more than 1% compared to the previous year. Only the proportion of animals used to maintain colonies of genetically modified laboratory animals decreased very sharply by around 55% compared to 2022.

Basic research

In 2022, as in previous years, prominent areas of basic research included, in particular, research into the immune system and the nervous system (21% and 19%), and the cardiovascular system (around 12%). As in the previous year, the 'other basic research' category continued to account for well below 1% (0.3% in 2023; around 1% in 2022) of all uses in the area of basic research. This reduction may be due largely to efforts by the competent authorities to scrutinise, on the advice of the European Commission, any use of 'other' categories.

Human and animal diseases

As in previous years, there was an emphasis on human cancers in the area of research into human and animal diseases. Some 39% of the animals used for testing in this area were used for this purpose.

Regulatory use and routine production

As in previous years, regulatory uses focused on toxicity and safety testing, including pharmacological testing. This sector accounted for almost 48% of reports within regulatory uses in 2023, representing a decrease of around 7% compared to 2022. At the same time, the proportion made up by quality checks (batch safety and potency testing) saw a moderate increase from around 19% in 2022 to around 23% in 2023. The proportion corresponding to routine production increased slightly to around 26% (around 24% in 2022). Around 56% of routine production could be ascribed to the production of antibodies (excluding the ascites method). This figure was around 7% lower than in 2022. In the 2023 reporting year, as in the previous year, no production of antibodies via the ascites method was reported.

(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 64% of uses). Around 27% of the tests were classified as 'moderate' and 3% were classified as 'severe'. The proportion of mild experiments decreased by around 2% compared to 2022 (66%). The proportion had previously increased in 2022, but this trend reversed in 2023. The proportion of moderate tests increased by around 3% compared to 2022. The proportion of severe tests reached its lowest level since 2018 at around 3%. The proportion of tests on animals carried out entirely under general anaesthesia and from which the animal never regained consciousness was around 5%, and therefore also at a very low level. Overall, however, the reported changes are small and within the usual year-on-year fluctuations in the distribution of severity levels.

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

Germany is keen to limit the number of animals used in testing to what is strictly necessary, and to ensure the best possible protection for laboratory animals. The German public expect more to be done to protect laboratory animals. For this reason, various projects are being launched and promoted at federal and Land level aimed at replacing animal testing with alternative methods as quickly as possible.

In 2015 the German Centre for the Protection of Laboratory Animals (Bf3R) was established at federal level by the Federal Ministry of Food and Agriculture (Bundesministerium für Ernährung und Landwirtschaft, BMEL), and since 2023 the Federal Government has had animal welfare officers. In 2022 the Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF) launched the 'Bundesnetzwerk 3R' [Federal 3R Network]. The aim of this initiative is to create a network for 3R [reduction, refinement and replacement] research and application in Germany. The focus is on inter- and transdisciplinary dialogue between academia, industry, politics, administration and stakeholder organisations. Various support measures are also available at federal level.

The support measures at federal level include the promotion of 'alternative methods to animal testing', the Foundation for the promotion of substitute and complementary methods to reduce animal testing (*Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen*, SET) and research funding from the Bf3R. In addition, the BMEL's animal welfare research prize is awarded annually in three prize categories, and the German Research Community (*Deutsche Forschungsgemeinschaft*, DFG) awards the Ursula M. Händel Animal Welfare Award every year.

Various measures have also been taken by the individual *Länder*. The number of centres dedicated to developing, researching and implementing alternative methods has increased significantly to 12 in total. Individual *Länder* also implement support measures and award research prizes for the successful development and research of alternative methods to animal testing. One such example is the Einstein Foundation Berlin. Since 2021 it has been supporting the Einstein Centre 3R with the aim of developing new therapies for human diseases while improving animal welfare.

The tireless efforts of the approval authorities in the respective *Länder* and of the animal welfare officers and welfare committees of various institutions in relation to the use of animals for testing purposes are also helping to reduce the number of laboratory animals.

The involvement of animal welfare organisations and foundations in a range of activities also plays a significant role in reducing the number of animals used in science.

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

'Other animal species' category

Overall, around 3% of all animals used were declared as 'other species' in 2023. The largest groups in this category were 'other fish' (around 62% of 'other' animals), 'other birds' (around 22%), 'other rodents' (around 5%) and 'other mammals' (around 5%).
In the 'other mammals' sub-category, mainly native wild animals, in particular bats, but also larger wild animals such as deer were reported, for which there is no separate category. 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) were used in particular. These species were used mainly in basic ethological research. This may have partially concerned animals caught in the wild that were released after the test was finished. In addition, zebra finches made up around 7% of reports, mainly for basic ethological research but also for research on the nervous system.

In the 'other fish' category, as in previous years, the use of fathead minnows (*Pimephales promelas*) for regulatory purposes, in particular ecotoxicity, has increasingly been reported. This species accounted for almost 15% of 'other fish'. In addition, around 11% of reports were for African turquoise killifish (*Nothobranchius furzeri*), which cannot be assigned to an existing category but are widely used in basic research and are also genetically modified. Finally, the 'other fish' sub-category also includes native wild fish (e.g. white fish, carp, perch and European eel), which were mainly used in basic ethological research. This may have mainly concerned fish caught in the wild that were released after the test was finished. The category 'sea bass (spp. from families e.g. *Serranidae*, *Moronidae*)', which was newly added in 2021, was not used in 2023 due to its narrow taxonomic scope.

In the 'other rodents' category, edible dormice (19%), naked mole-rats (18%) and multimammate mice (8%) were used in particular. While edible dormice were used in particular in the field of basic ethological research, naked mole-rats and multimammate mice were used in basic research on the immune and nervous systems. In addition, field mice, voles, bank voles and wood mice were also reported, which were used for different purposes. The use of field mice in authorisation procedures for plant protection products, which was still frequently reported in the 2022 reporting year, did not take place in significant numbers in 2023.

'Other uses' category

Overall, less than 1% of all animal testing was reported under 'other uses' in 2023. Around 50% came under the 'other basic research' subcategory and around 32% under the 'other regulatory purposes and other routine production' subcategory.
Within the 'other basic research' subcategory, there was particular emphasis on the following areas:

- research in the field of gerontology (around 54%);
- performance testing of livestock (around 18%);
- epigenetics (around 11%);

- molecular biology (around 4%).

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

'Other legislation' category

This category concerned only the efficacy testing of products under the Infection Protection Act (*Infektionsschutzgesetz*, IfSG).

(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 2023 reporting year, 26 cases were reported of pyrogenicity tests and eye or skin irritation tests under authorisation procedures for medicinal products, chemicals and medical devices. They involved 917 rabbits and 51 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 939 uses (around 97%) a substitute method was not or not yet recognised;
- in the case of 6 uses (less than 1%) the tests were necessary due to international requirements;
- in the case of 23 uses (around 2%), the reporting facility referred to the level of demand from clients, and the competent authority reported back that the use was plausible in these individual 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 2023, 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 2023 by approximately 14%. This is attributed to the diminished number of animals used by user establishments in Greece in relation to the projects authorised by competent authorities and completed in the year as well as to the fact that some establishments did not perform any protocols this year.

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

i) A decrease in the number of mice used in 2023 is noted compared to 2022 by approximately 5%.

Regarding rats, their number used is also decreased by 6% compared to 2022.

A significant decrease, more than 50%, in the number of rabbits used in 2023 compared to previous year. This is due to the fact that fewer number of establishments executed protocols that used rabbits compared to last reporting year.

A decrease in the use of pigs is noted by approximately 13%.

A 50% increase in the use of domestic fowls is noted compared to 2022 due to the number of domestic fowls that were re-used for "animal nutrition" purposes.

A significant decrease by 55% is also noted on animals used for "basic research" purposes compared to the previous reporting period while there is a 25% increase on animals used for "translational and applied research" purposes. These changes in the use of animals seem to be attributed to different focus on research from establishments between reporting periods.

A significant decrease by 90% in the use of zebra fish is noted due to the fact that some establishment used no zebra fish for 2023.

For the changes in statistics above where no other explanation is offered see point v. under section (b)

ii) Approximately the same proportions of genetically altered mice with a harmful phenotype is noted in Greek statistical data in 2023 compared to 2022 while not genetically altered mice are decreased by approximately 11% compared to 2022 and there is a slight decrease in the number of genetically altered mice without a harmful phenotype. Furthermore a significant decrease by approximately 38% is noted in basic research. This is attributed mainly to the significantly less number of zebra fish used for basic research during 2023. A significant increase by approximately 29% is noted in translational and applied research due to different focus on scientific research from establishments.

iii) A significant use of fish is still depicted in Greek statistical data in 2023 again compared to other MS for 2022 but lowered by approximately 30% compared to 2022. This is due to the fact that Greece is a Mediterranean country and has a number of user establishments dealing with studies on fish biology, behaviour/ethology and protection of natural enviroment. Sea bass and zebra fish are the main species used. In user establishments, fish are maintained under similar commercial production conditions, and most of the projects consist of variations in the rearing parameters (temperature, photoperiod, dissolved oxygen, tank size, feed type and frequency, rearing density, etc.) that may cause stress to the animals and are classified as "mild" apart from an establishment executing protocols classified as "severe" focusing on the protection of species.

iv) No cephalopods were used in 2023.

v) 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.

vi) 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.

vii) It has to be noted that the purpose of animals used for scientific purposes differs among each year according to the protocols authorised and funding received by user establishments. Minor changing trends can be recorded. For example in 2023 around 11% of protocols addressed Immune system basic research studies, 22% addressed protection of natural environment, 26% addressed protocols for translational and applied research related issues that mostly referred to human nervous and mental disorder protocols, human cancer and human immune disorders.

viii) 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.

ix) A continuous increase in the use of genetically altered animals is constantly noted during the last years, due to the type of projects authorised and the research trends of recent years globally. Their total number slightly decreases on 2023 compared to 2022 with the exception of genetically altered animals with a harmful phenotype where there is a slight increase in their number between 2022 and 2023.

x) A continuous decrease use of dogs and cats is noted during the years, according to the relevant protocols authorised. Their number has diminished between 2022 and 2023.

xi) Animals used for non-regulatory toxicology and ecotoxicology purposes were increased around 49% compared to 2022, due to the fact that one establishment executed more protocols this year compared to 2022.

xii) A significant decrease 40% in Toxicity and other safety testing including pharmacology was noted due to less animals required from the two establishments executing this kind of protocols in 2023.

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

i) Animals with "non recovery" severity seem to be slightly decreased by 2% approximately due to a decreased number of animals used for higher education purposes.

ii) Animals with "moderate" severity seem to be increased by 10% compared to 2022 due to the differentiation of protocols authorized for this year.

iii) The severity of the procedures is slightly decreased compared to 2022 as less severe protocols were authorised during 2023.

(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 major 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 2023 although with an increasing trend compared to 2022 due to more establishments executing protocols with other fish.

Greece remains a leading country in Mediterranean fish production and significant research is carried out in this field compared to other MS. Sparus aurata are the leading species, with Nile tilapia, to follow in general for 2023. 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 uses of animals for 2023.

(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 2023.

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 2023 was 110 799, which represents 8,3 % decrease compared to 2022. The reason is for the decreased use the 3R, with less animal uses for scientific purposes. The proportion of re-used animals was 3,56 % which increase compared to 2022 when was 2,29%.

The number of genetically altered animal used without a harmful phenotype in 2023 was 9 060, which represent about 3,7% increase compared with the previous year. In 2023 the percentage of use is 8,18 % from total uses.

The number of genetically altered animal used with a harmful phenotype in 2023 was 428, which represent 63.78% decrease compared with 2022. In 2023 the percentage of use is 0,39% from total uses. This also shows increase compare with 2022.

The newly acquired monkeys are all from self-sustaining colonies and from at least F2 generation.

(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 compared to previous years.

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

The number of uses of animals by severity decreased in mild from 67 719 to 55 218 and severe from 20 357 to 19 256. In case of moderate from 28 411 to 31 912 and non-recovery from 3 030 to 4 413 increased. Knowing the retrospective assessments, it can be assumed that partners report a severe classification for each animal in the licensed experiments with a severe classification, which may distort the data.

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

-

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

-

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

Animals were used in skin irritation and eye irritation/corrosion procedures because no clear results were obtained with other methods, but decrease can be observed.

(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 was no any cases.

Ireland

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

In 2023, the total number of animals used for research, testing, routine production, and education/training increased by 15% compared to 2022. Specifically, there were 107 873 uses of animals in 2023, up from 93 825 in 2022. Similarly, the number of animals used for the first time rose by 15%, with 106 639 animals used for the first time in 2023, compared to 92 939 in 2022.

Mice continue to be the most commonly used species, accounting for 81% of all animal uses. The total number of mice used increased by 29%, rising from 67 320 in 2022 to 86 871 in 2023. This increase was primarily due to a 39% rise in the number of mice used for regulatory testing in 2023, e.g. for the batch safety testing of human vaccines.

The number of rats used decreased by 36%, from 13 082 in 2022 to 8 440 in 2023. This decline was mainly due to a reduction in the use of rats for regulatory testing, specifically for batch potency testing of human medicinal (biological) products.

The number of guinea pigs used increased by 60%, from 741 in 2022 to 1 188 in 2023. This rise was driven by an increase in the use of guinea pigs in quality control and safety testing of human vaccine products.

The number of uses of rabbits used increased by 83%, from 1 012 in 2022 to 1 852 in 2023. This rise was due to an increased requirement for pyrogen testing as part of efforts to investigate and validate non-animal alternatives for detecting pyrogenic contaminants, in preparation for the removal of the rabbit pyrogen test from the European Pharmacopoeia in 2025.

The number of cats used for the first time in 2023 decreased by 75% compared to 2022, dropping from 32 to 8. Similarly, the number of dogs used for the first time fell by 52%, from 113 in 2022 to 54 in 2023. The factors underlying these reductions are not fully understood, but they may be due to lower volumes of research, development, and regulatory testing of veterinary medicinal products being undertaken during 2023 than in 2022.

The reported use of ferrets decreased by 20% in 2023 compared to 2022, with 175 ferrets used in 2023, down from 219 in 2022. This decline was due to a reduction in the use of ferrets for regulatory testing for influenza vaccines in 2023.

There was a significant decrease in the number of uses of horses reported for 2023 versus 2022 (10 uses of horses reported in 2023 compared to 219 uses reported for 2022). This was due to a large-scale study on equine metabolic disease concluding during 2022.

The number of reported uses of pigs decreased by 69% in 2023, with 752 uses compared to 2 460 in 2022. This decline was due to the conclusion of several large-scale studies on porcine nutrition and antimicrobial resistance in 2022.

The number of reported uses of domestic fowl decreased by 64% in 2023, with 37 uses compared to 102 in 2022. This reduction is due to the conclusion of a study aimed at improving chicken welfare in 2022.

The number of reported uses of *Xenopus* decreased by 59% in 2023, with 50 uses reported compared to 121 in 2022. This decline was due to a reduced usage of *Xenopus* larvae in translational and applied research on human sensory organs during 2023.

The number of reported uses of fish in the species category "salmon, trout, chars, and graylings" increased by 52% in 2023, rising from 1 364 in 2022 to 2 068. This increase was driven by studies conducted in 2023 aimed at conserving Irish salmon populations.

The number of reported uses of "other fish" increased by 200% in 2023, with 135 uses compared to 45 in 2022. This rise was driven by studies aimed at conserving various wild fish species whose populations are under threat.

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 28% from 2022. The reasons for this increase are not known.

The total number of animals used for 'Regulatory use and routine production' increased by 27% in 2023, with 75 109 reported uses compared to 59 245 in 2022. This rise was driven by an increase in the number of animals used for the secondary purpose 'Quality control'. The increase in numbers of animals used for 'Quality Control' testing resulted from increases in uses for batch safety testing of human vaccines, pyrogenicity testing of certain products intended for intravenous administration, and batch potency testing of certain biological medicines such as anti-venom products.

There was a 90% increase in the number of animals used for pyrogenicity testing (1 834 uses in 2023 versus 966 uses in 2022). This increase is as a result of sponsor companies requiring increased volumes of rabbit pyrogen testing as part of the process of investigating and validating non-animal alternative tests to detect pyrogenic contaminants, in preparation for the removal of the rabbit pyrogen test from the European Pharmacopoeia during 2025.

There was a 77% decrease in the number of animals used for the purpose 'Protection of the natural environment in the interests of the health or welfare of human beings or animals'. This was due to a large-scale study aimed at reducing methane emissions from sheep concluding during 2022.

There was a 75% decrease in the number of animals used for the purpose 'Preservation of species' (195 animals in 2023 versus 773 animals in 2022). This decrease was due to a large-scale conservation study of salmon species concluding during 2022.

There was a 25% decrease in the number of animals reported as used for the creation and maintenance of genetically altered animals. It is thought that this decrease is attributable to year-to-year variation.

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 2023 is largely similar to that of 2022, with no significant changes noted.

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

The HPRA has focussed significant efforts over the past number of years to ensure that there is a move to non-animal alternatives for regulatory testing, and this effort is reflected in the ongoing reduction in numbers of uses of animals for these types of tests noted over the last number of years. 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 61% reduction in the numbers of animals used for regulatory testing (75 109 animals used in 2023 versus 194 816 animals in 2017). The HPRA remains firmly committed to promoting the use of non-animal alternatives in both regulatory testing and biomedical research.

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

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

Furthermore, throughout 2023 we shared regular communications with end-users (quarterly newsletter as well as direct correspondence with relevant stakeholders) on 3Rs topics and developments, including on the EURL ECVAM reviews of advanced non-animal models in biomedical research, useful Reduction resources such as the NC3Rs EDA, and a wide range of Refinement resources.

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

With regards to species, only 152 animals in total were reported as ‘other’ species, accounting for 0.14% of total animal use. This relates to wildlife/marine-life conservation studies using e.g. wild squirrels and fish species such as shark and tuna species.

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.

1 834 rabbits were used for pyrogenicity testing in 2023. Alternative testing methods to determine the pyrogenicity of medicinal products are recognised under the legislation of the Union. However, the use of these methods for certain classes of medicinal products (e.g., some blood/protein-based medicines) poses challenges. Therefore, in order to meet the necessary regulatory safety requirements for release of certain medicinal products onto the market there is currently no alternative to the use of the rabbit pyrogen test. However, sponsor companies are engaged in validating non-animal alternative tests and gaining regulatory acceptance for these.

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

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

- **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**:

This was not exceeded during 2023.

Italy

The data for Italy for 2023 come from the Ministry of Health – Directorate-General for Animal Health – Office 6 – Animal Welfare, Animal Hygiene and Urban Veterinary Hygiene.
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 has been a general fall in the number of naïve animals involved (from 548 933 in 2019 to 365 130 in 2023). The decrease may have been caused, in part, by restrictions resulting from the COVID-19 health emergency (over the years 2020-2021), and partly by the use of alternative methods to the use of animals for certain toxicity tests, in accordance with national and European (regulatory) legislation, such as: LAL tests and MAT tests. Also in a regulatory context, new in silico and in vitro models for immunogenicity tests for abnormal toxicity have been used to test medical devices (ISO10993-10). In vitro tests for the detection of algal biotoxins, for the diagnosis of rabies and botulism are used for disease diagnosis.

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

An increase in the number of naïve zebra fish (Danio rerio) can be seen. These were used mainly in basic research in the fields of oncology and the study of nervous diseases.

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

As far as 'serious' and 'moderate' levels of suffering are concerned, the rate remains below 30% for both levels. We would reiterate that more deliberate use by researchers of tools such as clinical assessment sheets that record the severity of procedures is contributing to a more appropriate classification of levels of suffering.

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

The animal welfare bodies have been running awareness-raising activities on checking the application of the 3Rs principle in the evaluation of research projects before the reasoned opinion is issued, which is essential for the approval of a research project.

The principle of the 3Rs is addressed in the basic training of operators and in their ongoing professional development.

The above activities are contributing, 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:

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

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

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

Rabbits are used for **pyrogenicity** tests for the release of batches of medicines intended for certain non-EU countries (USA and other countries) and for the release of medical devices.

Skin sensitisation and skin irritation tests are used to test medical devices, as required by OECD Guideline 406, or performed in accordance with ISO 10993-10; ISO 10993-23 and Local Lymph Node Assay (LLNA) test.

Eye irritation/corrosion tests under ISO 10993-23 on medical devices are also reported.

(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**:

None**.**

Latvia

This report does not include data on the use of animals for the creation and maintenance of genetically modified animals.

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

The competent authority approved 9 pilot projects in 2021, 10 in 2022 and 12 in 2023. The total number of animals used for scientific purposes has declined in recent years – 3 452 were used in 2021, 2 114 in 2022 and 1 922 in 2023. Since it generally takes more than one calendar year to design a pilot project, most of the pilot projects authorised in preceding years, especially towards the end of the year, are implemented in a subsequent period. Thus, taking all authorisations issued in previous years into consideration, authorisations were active for 35 pilot projects in 2021, 42 in 2022 and 41 in 2023. As in previous years, for various reasons, animals were not used in all pilot projects for which authorisations had been issued, nor were all authorised pilot projects actively pursued. Most pilot projects involve several procedures, so often, depending on the design of the pilot project, some of the procedures may be implemented within a single year and some successively over the course of the entire project. This does not mean, however, that all procedures were carried out for all projects and that all projects were implemented as planned. In some cases, projects or procedures were temporarily suspended owing to a lack of funding or because no additional *in vivo* studies were carried out prior to clinical trials (studies) because reliable and acceptable data had already been obtained using new alternative methods not yet available when the pilot project was initially planned or because the first *in vivo* tests of newly synthesised substances demonstrated that the further use of animals would be superfluous because the new substances being investigated had proven to be ineffective and did not demonstrate the sought-after characteristics. Science is advancing rapidly from year to year, so researchers use *in vitro* methods as much as possible once new information in their field of research is obtained, leading to a reduction in the overall number of animals used. In most cases, especially where long-term projects (of five years' duration) are concerned, researchers use fewer animals than indicated in the project design.

A total of 281 genetically modified animals were used in 2020 and 224 in 2021. The number of genetically modified animals used in procedures continued to grow in subsequent years, reaching 309 in 2022 and 333 in 2023. The use of animals with harmful genotypes has also slightly declined (n=104 in 2021, n=47 in 2022 and n=9 in 2003). This trend is due to the fact that projects in which genetically modified animals have been actively used have been completed in prior years. The percentage share of the total number of animals used in procedures which is represented by genetically modified animals has increased significantly: 7.3% in 2020, 6.5% in 2021, 14.6% in 2022 and 17.3% in 2023. The increase in the percentage of genetically modified animals relative to the total number of animals used in procedures can be explained by the need for a pathological model to evaluate new substances with potential therapeutic effects and new therapeutic methods. Science is constantly evolving, but currently it is often less harmful to assess the effectiveness of a particular substance or treatment method using genetically modified animals than to establish a pathological model (e.g. use a specific diet or surgical method to induce a pathological condition). In most cases it is possible, using genetically modified animals, to create and use in research the early stages of a disease or pathological condition for which clinical manifestations cannot yet be observed, but which can be detected only using specific diagnostic methods, devices or analyses (tests) that cause animals much less discomfort and harm than the full course of development of the disease or the induction of a pathological condition. Consequently animals are subjected to lighter and shorter procedures. Furthermore, in some cases where animals are used as models for

research into vaccines and immunological cancer treatments, a model is needed which is very similar to a human being and fulfils specific conditions (e.g. has cell receptors which are identically expressed). In such cases, it is often possible to replace higher-sensitivity species with lower-sensitivity species (e.g. mice or rats may be used instead of primates).

This year (2023) there was no reuse of animals in procedures performed during *in vivo* studies.

Most of the animals used in procedures are of EU origin (92.45%) and the majority – 65.8% – are from registered test animal breeders. For various reasons (see Table 1) animals are also obtained from other parts of the world, including from unregistered test animal breeders.

Table 1

Animals not born at a registered test animal breeder			
Region	**Species**	**Count**	**Explanation of need**
America	Mice	30	Special animal strains not available to breeders in European and EU countries.
European Union countries	Mice	225	Animals bred in a test animal user's undertaking for use in their specific procedure.
	pigs	8	Irreversible procedure (acquisition, maintenance or upgrading of higher education or professional skills)
	cats	334	Epidemiological and fundamental research
	Sheep	44	Translational research (cardiology)
	cattle	2	Field studies (fundamental research/ethology/animal biology)

In 2023 the number of animals used in procedures and not born at a registered test animal breeder in the EU increased significantly, from 5.5% in 2022 to 29.9% in 2023, which can be explained by the fact that in 2023 the projects pursued included those which had been authorised in previous years and involved farmed animals, and by the tendency among some test animal users, for various reasons, to breed rats and mice on their business premises for research purposes. These undertakings have therefore registered solely as test animal users. The number of animals used in procedures born outside the territory of Europe has also increased slightly, from 2.0% in 2022 to 4.4% in 2023. By contrast, no animals were used in procedures in 2023 which had been born in Europe but not in EU Member States.

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

A comparison of data on animals used in procedures over the last five years shows that the total number of mice used decreased from 3 078 in 2019 to 1 281 in 2023. As Figure 1 shows, fewer mice are being used every year. The main reason for this is constant scientific progress and ever more careful planning of procedures, with a progressive increase in the use of *in vitro* methods.

The total number of rats used in experiments is also tending to decline (see Figure 1). Sometimes, in isolated studies (mainly those involving the testing of new substances), rats are chosen rather than mice because of their larger size. This characteristic enables researchers to obtain more biological matter (e.g. tissues, blood samples or tumour cells) for further *in vitro* testing. This

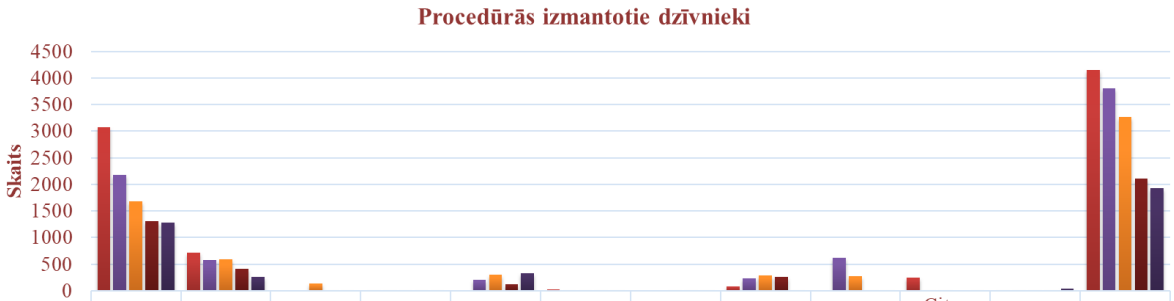
makes it possible to obtain all the necessary information and carry out the study while at the same time using fewer animals in procedures.

The number of pigs used declined slightly this year, from 10 in 2022 to 8 in 2023. Since these animals are used in only one procedure (for the acquisition of higher education or the acquisition, maintenance or upgrading of professional skills), the decrease is mainly due to the specificities of project implementation in the year in question.

Following the conclusion of projects approved in previous years involving research on wild birds, no new experiments on wild birds were begun in 2023.

The number of cats used in procedures increased sharply in 2023. In 2022, the number of cats used was 119 (6.4%), whereas in 2023 it reached 134 (17.4%). The research methodology was approved in 2022 and studies were carried out in 2023. Animals were subjected to light procedures, mainly as part of epidemiological studies (coronavirus and COVID-19-related studies) and ocular physiological studies. The harm done to these animals during the procedures was associated with the removal of blood, nasal discharge, and tear samples. After sampling, all these animals were immediately returned to their owners. In most cases, the owners themselves were present when the animals were subjected to the procedures, and often procedures were carried out in housing to reduce stress and discomfort for the animals.

Figure 1



	Peles	Žurkas	Kāmji	Truši	Kaķi	Cūkas	Liellopi	Citi putni	Sikspārņi	Citas zivis	Aitas	Kopā
2019	3078	717		6		24		80		240	0	4145
2020	2178	578		2	198	10		232	614	0	0	3812
2021	1678	584	132	12	299	7	2	284	270	0	0	3268
2022	1314	410	0	0	119	10	0	257	0		4	2114
2023	1281	256	0	0	334	8	2	0	0	0	41	1922

As described above, the surgical devices for carrying out the studies were approved in 2022; two sheep were used at that time (in 0.2% of procedures). The study (relating to the practical surgical treatment of cardiovascular diseases) began to be implemented in 2023; 41 sheep were subjected to procedures then (accounting for 2.1% of the procedures).

Mention should also be made of cases where authorisation for performing procedures has been granted for several years at a time (permits are often issued towards the end of a particular year, with all the animals being used for research in the following year). Exceptional situations sometimes tend to arise in which problems obtaining funding are encountered and implementation of the project in question is slightly delayed, resulting in animals being used after the validity period of an authorisation for a project has expired. Checks have not, however, revealed that more animals are being used in procedures deliberately than initially envisaged. On the contrary: in most cases when approving the devices needed for a particular study, the conclusion has been reached that it is possible to use fewer animals than initially planned, as a result of which adjustments have been made to the project methodology. Situations are also often observed in

which medicinal substances or vaccine candidates do not prove to be as effective as desired and are excluded from further research stages, which allows for the number of test animals used in procedures to be reduced even further.

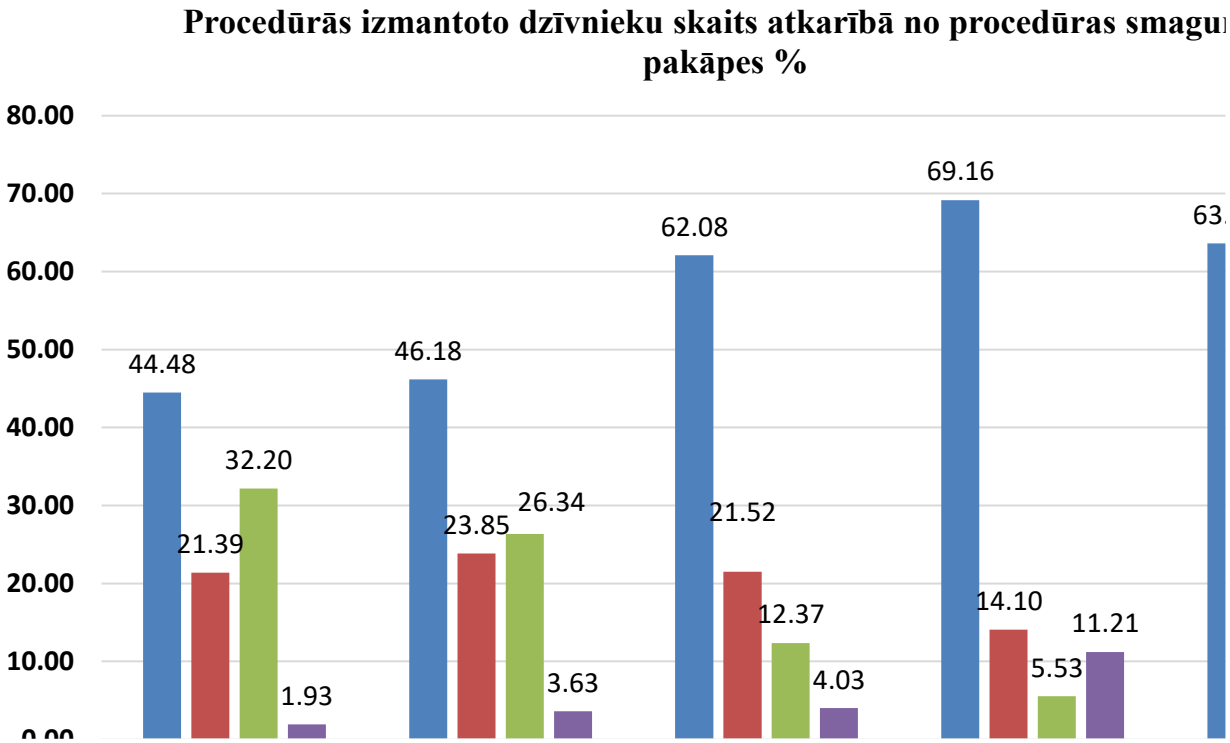
(c) Information on any changes in trends in actual severities and analysis of the reasons thereof:

The vast majority (63.6%, n = 1 223) of all test animals were subject to **light procedures** in 2023 (see Figures 2 and 3) – of these, 151 were used in planned light procedures.

- 35 rats (objective of the pilot project - Translational and applied research: Non-regulatory toxicology and ecotoxicology) were subjected to a light procedure rather than a moderate procedure, as these animals were used solely as the control group and were not subjected to manipulations of moderate severity.
- 16 mice (objective of the pilot project - Translational and applied research: Human cancer) were subjected to a light procedure rather than a moderate procedure, as these animals did not display any side effects elicited by the substance under investigation.
- 12 mice (objective of the pilot project - Translational and applied research: Human cancer) were subjected to a light procedure rather than a moderate procedure, as these animals were used solely as the control group and were not subjected to manipulations of moderate severity.
- 9 mice (objective of the pilot project - Fundamental research: Gastrointestinal system including liver) were subjected to a light procedure rather than a moderate procedure, as these animals were used solely as the control group and were not subjected to manipulations of moderate severity.
- 9 mice (objective of the pilot project - Human urogenital/reproductive disorders) were subjected to a light procedure rather than a moderate procedure, as these animals did not display any side effects elicited by the substance under investigation.

In 2023, a total of 108 animals (5.6%) were subjected to **severe procedures**; of these, 83 mice and 11 sheep were subjected to a planned severe procedure, whereas the rest endured severe suffering due to unforeseen circumstances. During a planned moderate procedure (objective of the pilot project - Fundamental research: Nervous system) three rats inexplicably did not regain consciousness after being operated on and were humanely euthanised.

Figure 2



In 2023, 576 animals (29.9%) were used in **moderate procedures**; of these, 477 mice, 97 rats and two bovine animals were subjected to planned moderately severe procedures.

In 2022, 15 animals (0.8%) were used in **irreversible procedures**. Of these, eight (pigs) were used in planned irreversible procedures, whereas seven (mice) subjected to planned moderate procedures involving surgical intervention (objective of the pilot project - Fundamental research: Nervous system) inexplicably failed to regain consciousness after anaesthesia and died.

A comparison of the number of animals subjected to procedures in 2022 and 2023 shows that the number of animals subjected to moderate procedures was bigger in 2023 (up from 298 (accounting for 14.1% of the total number of animals subjected to procedures in 2022) to 576 (accounting for 29.97% of the total number of animals subjected to procedures), which can be explained by the fact that, in the light of scientific progress, the attempts made to replace severe procedures with more humane methods and the administration to animals of various authorised adjunctive therapies, a decline in the share of severe procedures, depending on the type and purpose of the study in question, has been achieved, with the share declining from 11.2% in 2022 to 5.63% in 2023. Similarly, over the course of the last four years, a decline in the number of animals subjected to irreversible procedures has been achieved, from 26.0% in 2020 to 0.8% in 2023. A comparison of the research methods used in previous years showed that *in vitro* methods are being actively introduced wherever research specifications allow. For example, in cases where tests are carried out to ascertain the toxicity of various substances on cell cultures and isolated organs and tissues are employed in tests on newly synthesised substances, which enables *in vivo* research with irreversible effects to be replaced.

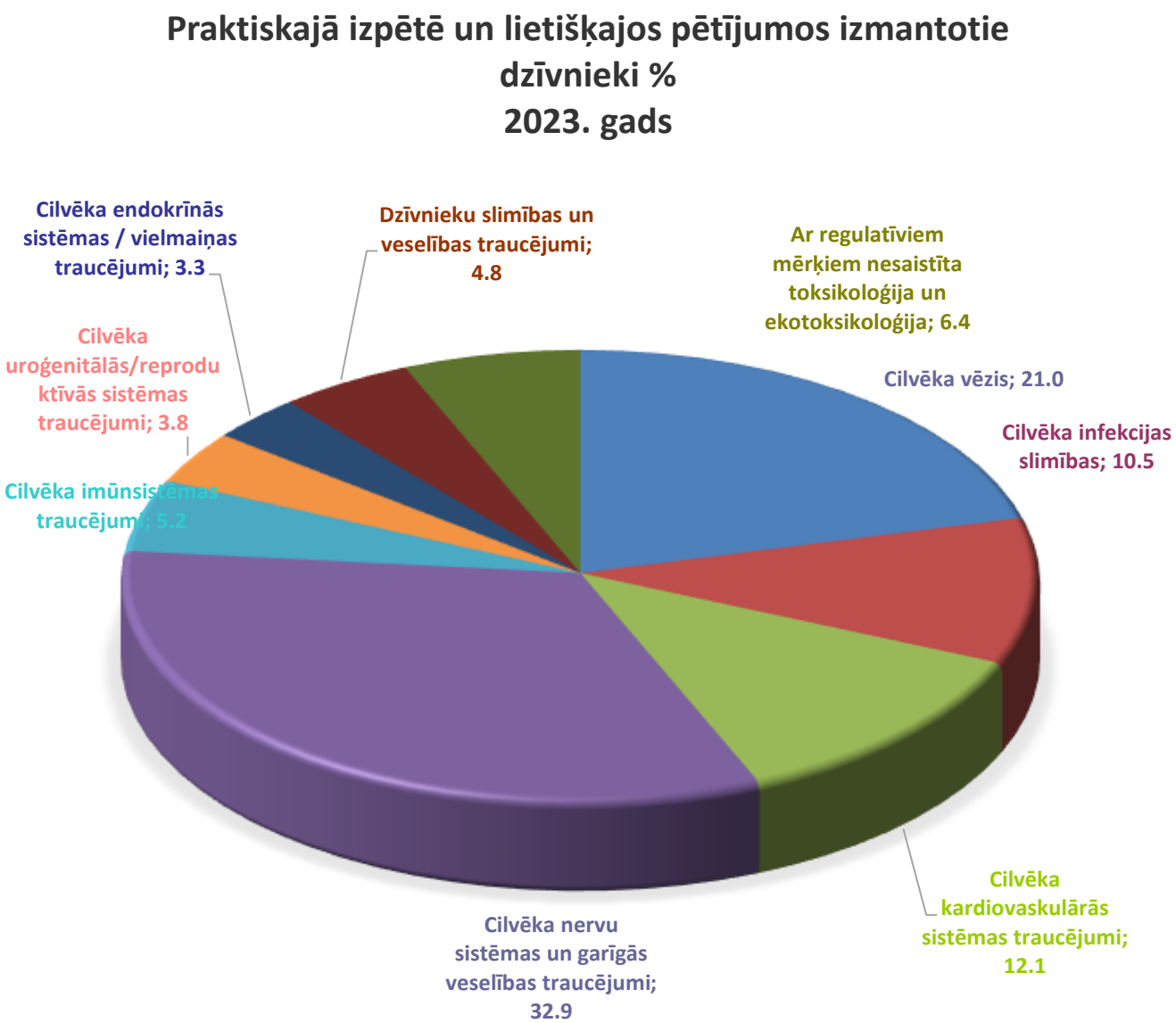
The total number of light procedures performed has nevertheless declined slightly, from 69.2% in 2022 to 63.6% in 2023. This is linked to trends in practical cancer research and research into new medicinal substances and vaccines for the treatment of various serious and complex illnesses (neurological diseases, diseases of the alimentary canal and diseases of the metabolism) which, for the purposes of researching potential medicinal substances, need to be introduced into animals

that are already clinically ill. Since these diseases have a severe effect on humans, efforts are made to elicit as similar a pathological condition as possible in the test animals in order to enable an assessment to be made of the effectiveness of any potential new medicinal substances.

The main research strand is ‘translational and applied research,’ for which 702 animals (36.5%) have been used, and ‘fundamental research’, for which 1 212 (63.1%) have been used.

Research within the 'translational and applied research’ strand has related predominantly to the human nervous system and mental disorders, with 139 animals (32.9%) having been used for this (see Figure 3), whereas 255 animals (21.0%) have been used for research into human cancer and 147 animals (12.1%) have been used for research into human cardiovascular disorders.

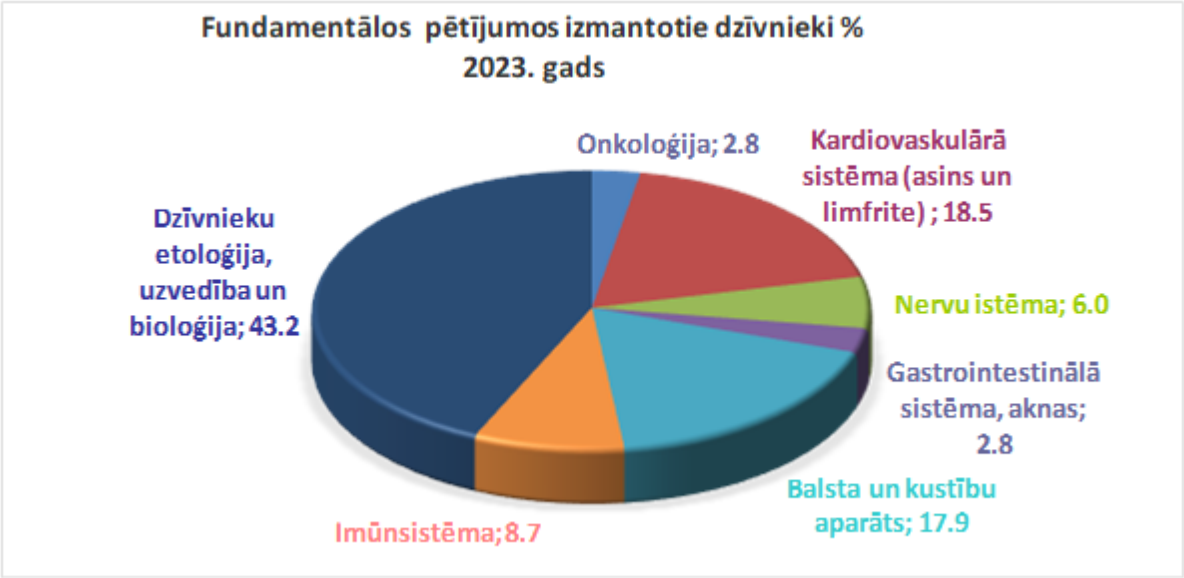
Figure 3



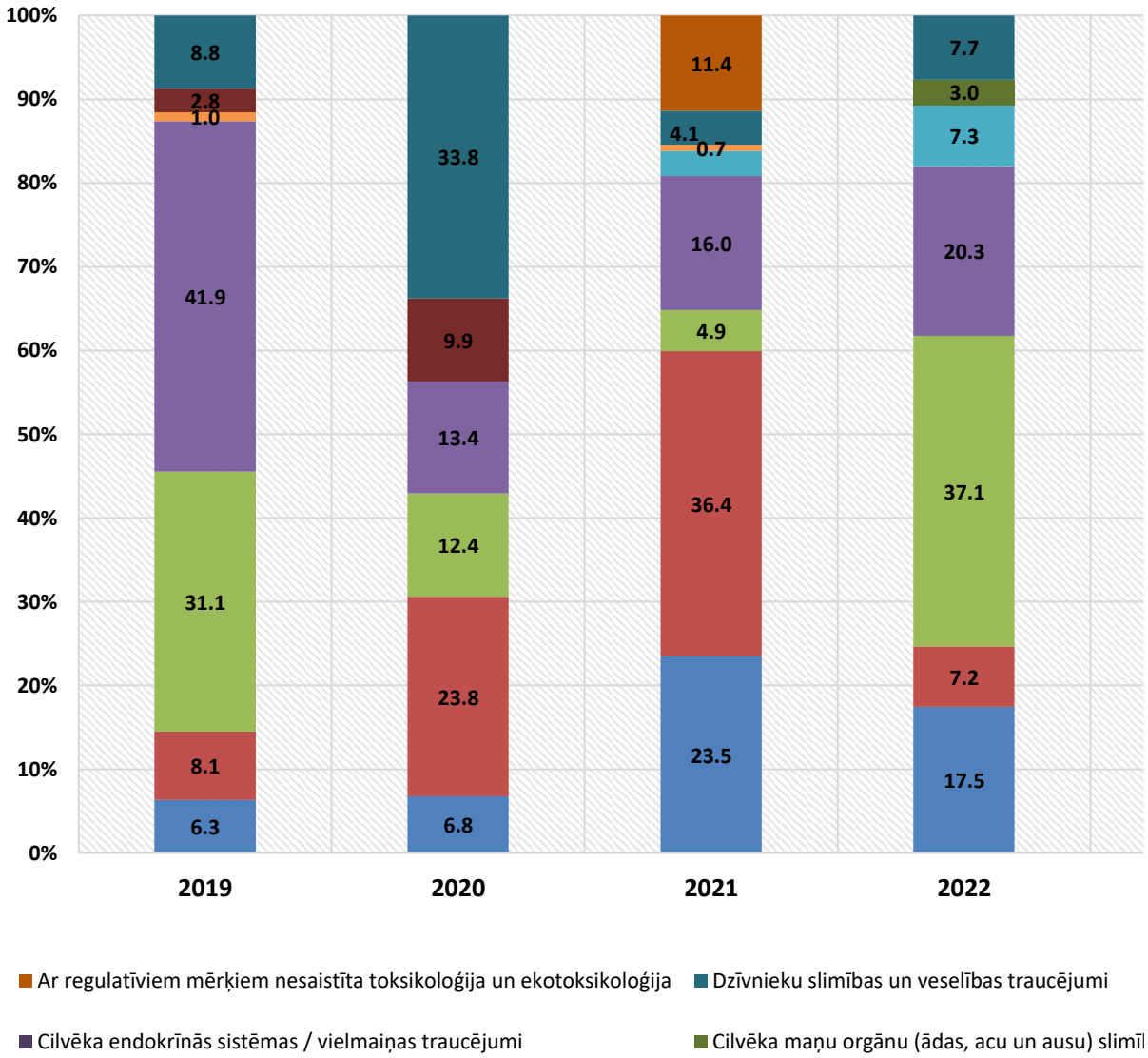
The largest proportion of studies conducted in the area of translational and applied research in 2023 pertained to human nervous system and mental disorders (32.9%). This, with few changes, has also been one of the most researched topics in all preceding years. A similar situation can also be observed with regard to research into the human cardiovascular system. The proportion of studies conducted into this topic has varied over the years, with the highest proportions attained in 2019 (31.1%) and 2022 (37.1%). By contrast, declines in the number of animals used in studies

were observed in 2020 and 2021 (12.4% and 12.1% respectively). The above fluctuations in the proportion of studies can be explained by the fact that research was conducted most often into various potentially new medicinal substances which are not subjected to *in vivo* effectiveness tests. Often pilot projects are conducted initially with a scrupulously approved research methodology to ensure that the number of animals subsequently subjected to tests is kept to a minimum and the exposure time and severity of harm done to those animals is reduced. Barring a minor discrepancy in 2022, the proportion of studies conducted into human cancer has been exhibiting an upward trend (from 6.3% in 2019 to 21.0% in 2023). Latvian scientists are conducting research into this area to find new vaccines to combat various aggressive cancers. Most research is aimed at finding new methods for treating breast cancer.

Figure 4

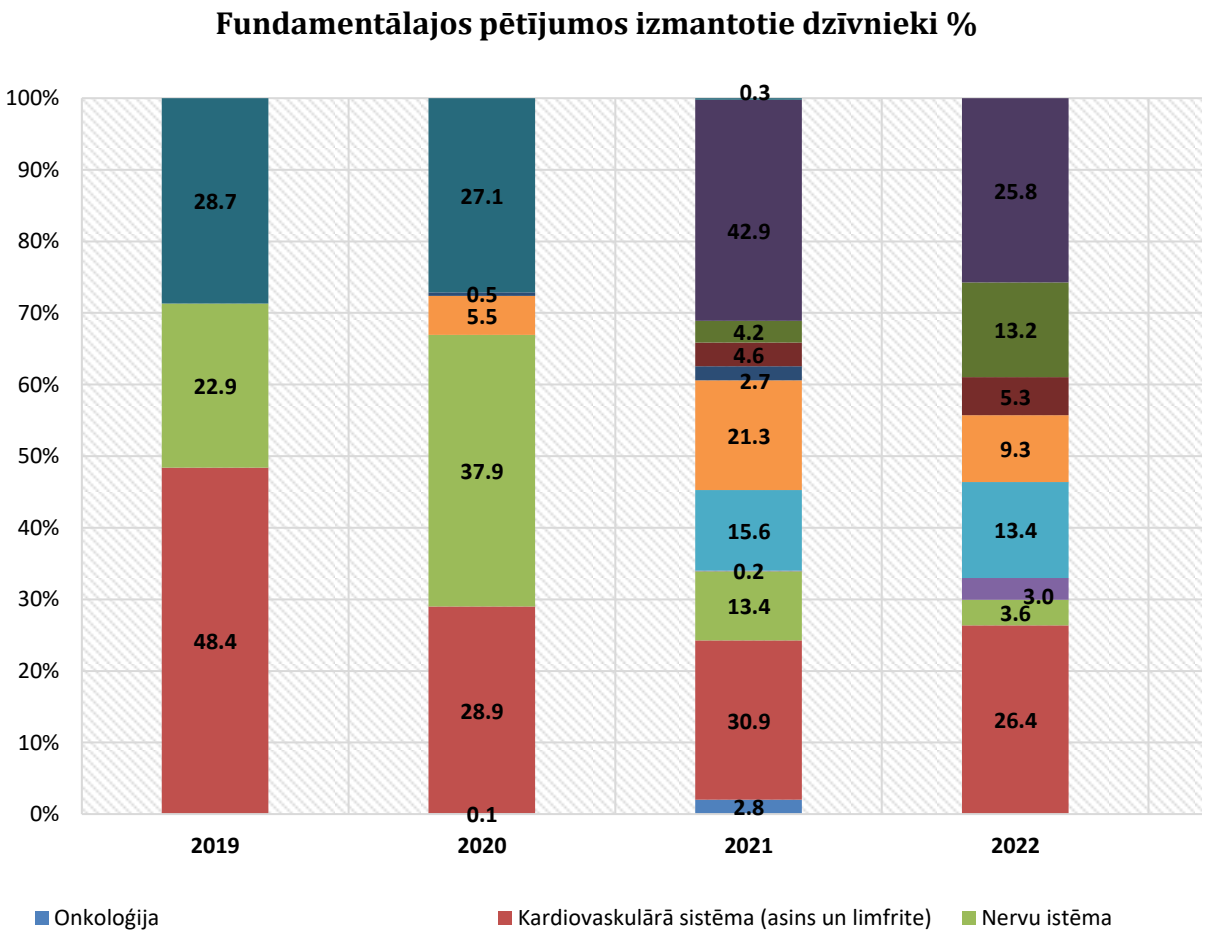


Praktiskajā izpētē un lietiškajos pētījumos izmantotie dzīvnieki %



In the domain of fundamental research, the most work has been done on ethology, animal behaviour and biology, with 303 animals (43.2%) having been subjected to procedures (see Figure 4), and also on the cardiovascular blood and lymphatic system, with 130 animals (18.5%) subjected to procedures, and on the musculoskeletal system, with 125 animals (17.9%) subjected to procedures.

Figure 5



As can be seen from Figure 5, fundamental research is being performed most often into the cardiovascular system. There has nevertheless been a downward trend in the share of studies being conducted into this topic over the last few years (from 48.4% in 2019 to 18.5% in 2023). By contrast, the share of studies being conducted within the second most popular domain - ethology and animal behaviour and biology - increased in 2023, reaching as high as 43.2%.

These trends can be explained by the active implementation by test animal users of projects relating to the behaviour and biology of both wild and domestic animals, and the increase in the number of studies in that domain. Research is being conducted on individuals of species of bats, red deer, migratory birds living in the wild and cats. Over the last three years the amount of research into the musculoskeletal system has also increased (from 15.6% of studies in 2021 to 17.9% in 2023), whereas the proportion of research being conducted into the nervous system has declined (from 22.9% in 2019 to 6.0% in 2023).

When carrying out an assessment of the pilot projects, experts representing the relevant scientific fields perform a detailed assessment of the methodology used in those projects and pay close attention to the reasons for choosing alternative methods and the in vitro studies carried out prior to *in vivo* testing. The rationale for choosing the number of animals and species is also assessed, and testers are increasingly being encouraged to assess not only the need to use animals in studies, but also the need for each manipulation and the possibility of minimising the suffering of animals during procedures. Therefore, as a result of systematic and careful work, there is an increasing downward trend in the number of animals used for experimental purposes year-on-year. By contrast, the number of authorisations issued for pilot projects is increasing.

Similarly, in their ongoing scientific work, researchers are looking for new alternative methods and ways to minimise the use of animals in procedures, and the recommendations of the project evaluation committee with regard to the 3RS principles are also being taken into account. Project authors are also seeking to increase the use of *in vitro*, *in silico* and *ex vivo* methods (e.g. use isolated organs, cells or organelles instead of live animals), in particular in first-stage toxicity and efficacy tests. Scientists also use organs and tissues from animals that were used in other light procedures as control group animals.

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

Authors of scientific projects are seeking to use *in silico*, *in vitro* and *ex vivo* methods in substance testing to determine the most efficacious test component before resorting to the use of animals. A literature review is also carried out and cooperation undertaken with other scientists conducting similar studies. Additionally other equivalent research is used for data comparison and analysis in order to avoid, as far as possible, duplication of research and to reduce the use of animals in procedures. When assessing a pilot project, the competent authority and experts ensure and verify the scientific feasibility and benefits of the project, analyse the possibility of replacing animals with alternative methods and assess the number of animals required for procedures, specific research methods and tests. The competent authority and experts also verify whether it is possible to attain the project's objectives as set out in the project plan. If it is found that animal suffering or the total number of animals used in procedures can be reduced, applicants are instructed to make the relevant changes to the pilot project before authorisation is granted. Furthermore, when inspections are carried out at test animal user undertakings, each project is checked for compliance with its approved methodology.

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

In 2023, no animals were used in procedures involving 'other animals'. This can be explained by the fact that the pilot projects involving research on birds living in the wild (common starlings (*Sturnus vulgaris*)) were completed in the preceding years.

(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 precedents for the use of animals in such categories have been identified.

(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:** No permits were issued for pilot projects involving procedures that might exceed the 'severe' classification threshold. No information was received from users in 2023 that a 'severe' classification had been exceeded in any procedure.
- **Details of the use:**
- **Reasons why the 'severe' classification was exceeded:**

No such cases were detected in 2023.

Lithuania

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

Compared to the number of animals consumed in 2022, which was 6 174, the number of animals consumed in 2023 was 4 936. This means that the total number of animals consumed decreased by ~20% (minus 1 238).

The number of users:

YEAR	NUMBER OF ANIMALS	THE NUMBER OF USERS
2013	3450	8
2014	2351	12
2015	2451	12
2016	2660	14
2017	2766	14
2018	3286	15
2019	5066	15
2020	3788	15
2021	5802	16
2022	6174	15
2023	**4936**	15 (only 10 users are active)

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

The Basic Research targets were the highest number of animals consumed in 2023, i.e. 2 990. Compared to the 2022 data, the use of animals for this objective decreased from 3,723 to 2 990 (a decrease of 733 units or 20%).

The number of animals used for Trans/Appl Research purposes remains similar, with a downward trend, i.e. 1 695. Compared to the 2022 data, the number of animals used for this purpose decreased from 1 776 to 1 695 (a decrease of 81 units or 5.5%).

The number of animals used for Higher education purposes has decreased sharply, i.e. 74. Compared to 2022 data, the number of animals used for this purpose decreased from 560 to 74 (a decrease of 486 units or 87%).

Regulatory use/Toxicity and the number of animals used for this purpose is 177. Compared to the 2022 data, the number of animals used for this purpose increased from 115 to 177 (an increase of 62 units or almost 30%).

Basic Research	[PB1] (Basic Research) Oncology – **622** [PB2] (Basic Research) Cardiovascular Blood and Lymphatic System – **250** [PB3] (Basic Research) Nervous System – **182** [PB5] (Basic Research) Gastrointestinal System including Liver – **105** [PB6] (Basic Research) Musculoskeletal System – **95** [PB7] (Basic Research) Immune System – **81** [PB8] (Basic Research) Urogenital/Reproductive System – **2** [PB9] (Basic Research) Sensory Organs (skin, eyes and ears) – **1430** [PB12] (Basic Research) Ethology / Animal Behaviour /Animal Biology – **50** [PB14] (Basic Research) Developmental Biology – **173**	**2990**
Trans/Appl Research	[PT21] (Trans/Appl Research) Human Cancer – **310** [PT23] (Trans/Appl Research) Human Cardiovascular Disorders – **58** [PT24] (Trans/Appl Research) Human Nervous and Mental Disorders – **417** [PT26] (Trans/Appl Research) Human Gastrointestinal Disorders including Liver – **145** [PT27] (Trans/Appl Research) Human Musculoskeletal Disorders – **11** [PT28] (Trans/Appl Research) Human Immune Disorders – **49**	**1695**

	[PT29] (Trans/Appl Research) Human Urogenital/Reproductive Disorders – **43** [PT30] (Trans/Appl Research) Human Sensory Organ Disorders (skin, eyes and ears) – **332** [PT31] (Trans/Appl Research) Human Endocrine/Metabolism Disorders – **275** [PT37] (Trans/Appl Research) Non-regulatory toxicology and ecotoxicology – **55**	
Higher education	[PE42-1] Higher education – **74**	**74**
Regulatory use/Toxicity and..	[PR81] (Regulatory use/Toxicity and../Acute toxicity) LD50, LC50 – **152** [PR83] (Regulatory use/Toxicity and../Acute toxicity) Non lethal methods – **10** [PR85] (Regulatory use/Toxicity and..) Skin sensitisation – **15**	**177**

Some approved establishments did not perform any projects in 2023 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 are used in the procedure classified as mild [up to and including], with 3 035 animals or 62% of the total used in 2023. Compared to the 2022 data (69%), the proportion of animals used in the mild [up to and including] procedure in 2023 was 7% less.
The total number of animals used in the Moderate procedure in 2023 was 1 380 animals or 28%. The percentage of animals used in the Moderate procedure has decreased compared to the 2022 data, i.e. 13% less than in 2022.

For the non-recovery procedure, 403 animals or 8% of the total, were used in 2023. The percentage of animals used in the non-recovery procedure increased by 4% compared to the 2022 data.

The number of animals used for the severe procedure was 118 in 2023 or 2% of the total number of animals used. Compared to the 2022 data (severe ~1%), the number of animals used for severe procedure has increased from 42 to 118 or 2 percent of the total number of animals used in severe procedure.

The 'severe' classification reported in 2023 consists of 118 animals: Mice (Mus musculus) 86, Rats (Rattus norvegicus) 26, Rabbits (Oryctolagus cuniculus) 6.

In one project "The evaluation of the treatment efficiency of electromagnetoporation combined with chemicals and dendritic cell vaccines for the treatment of mouse tumors", tumour cells were injected as part of a planned procedure to form a tumour. 1 mouse grew a larger tumour than the other mices in the control group. The mouse was euthanised after assessment of the current dynamics of tumour expansion.

Other animals were used in severe procedures, were killed or found dead after anaesthesia in these projects:

- "Studies on the toxicity, pharmacokinetics and pharmacodynamics of the latest medicinal products in various models of eye disease in mice and rats that have been developed" – 8 Mice (Mus musculus); 22 Rats (Rattus norvegicus);
- "Effectiveness studies of newly developed and already marketed drugs using models of diabetic retinopathy" – 5 Mice (Mus musculus); 4 Rats (Rattus norvegicus);
- "Study of the latest drug candidates using innovative non-invasive in vivo imaging models enabled by ocular disease in experimental rabbits" – 6 Rabbits (Oryctolagus cuniculus);
- "In silico chemogenomics-based toxicity studies of highly potent compounds against S. aureus" – 72 Mice (Mus musculus).

(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.

In addition, it should be noted that the National Contact Point of the Competent Authority shares all relevant information from the European Commission's meetings with the experimental animal establishments on the enforcement and implementation of the 3Rs principles.

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

In the category Animal species [A29] Other birds (other Aves) 21 Eurasian siskin (Spinus spinus) and 40 Atlantic canary (Serinus canaria) animals were used for [PB7] (Basic Research) Immune System purposes during the reporting in 2023.

(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 involving the use of live animals for purposes for which an alternative, non-animal method, is recognised by the legislation of the Union.

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

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

No such cases in 2023.

Luxembourg

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

In Luxembourg we could observe a general decrease in the total number of animals used in procedures from 25 293 animal in 2017, 13 751 animals in 2018, 11 117 animals in 2019, 5 341 animals in 2020, 4 967 animals in 2021, 3 601 animals in 2022 to 3 196 in 2023. This decrease may be a consequence of the reduction of the animal research activity and the application of the replacement and reduction.

Concerning the distribution in the species, the main reduction is due to a decreased use in mice and zebrafishes in procedures, notably in 2023: 3 037 mice were used compared to 3 201 mice used in 2022, and 123 zebrafishes compared to 400 in 2022.

Regarding the purpose of the animal uses, no trends were observed during the last year. The main category is basic research, followed by translational and applied research and training.

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

In Luxembourg, a constant decrease in the total number of uses and in the total number of uses in mice could be observed in the last years. This decrease may be a consequence of the reduction of the animal research activity and the application of the replacement and reduction.

Furthermore, in Luxembourg two main institutions are involved in animal testing. Both modernised. Due to the small number of the parties involved in animal experiments (3 facilities in total), the development of the animal facilities has a strong impact on the total number of animals used.

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

Comparing the actual severities during the last years, no significant changes have been observed.

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

The particular efforts taken to promote the principle of the Three Rs have been:

- The competent authority organised a second edition of a 3 R symposium in 2022, where all the members of the animal welfare bodies and users participated focusing on the replacement, reduction and refinement.

- During the inspections 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. Furthermore attention is paid to avoid the breeding of surplus animals.

- Replacement - alternatives to animal experiments whenever possible: investing in and supporting the development of alternative research methods. The initiatives include promoting human-based studies, encouraging the use of in vitro methods to gather preliminary data, and collaborating with other research institutions to share best practices. For instance, advanced in vitro techniques like organoids (derived from animals and patients) or organ-on-chip technology enable the creation of miniature tumors or organs, providing valuable insights while significantly reducing the need for live animal testing. The Luxembourg Research Institute has put significant effort into developing innovative animal-free alternatives, such as tumor organoids—three-dimensional tissue cultures derived from patient tumor that mimic the specific features of the patient's cancer. For the setup of imaging protocols and training for our magnetic resonance imaging (MRI) system, "mouse phantoms" are used to replace live animals. Additionally, Luxembourg institutes organize Function A and B training, where, to replace animals at the early stages of training, a full-day session is conducted using dummies for practical work.

- Reduction - the number of animals used in research are minimized by carefully designing experiments that require fewer animals while still achieving scientific objectives. There are statistical tools used like Ginger to optimize study designs, ensuring that the fewest animals necessary are used (https://clinicalbiometrics.shinyapps.io/GINGER/).

Comprehensive services (full service) are set up to help to decrease user variability, thus reducing the number of mice per group.

Single colonies of wild-type individuals (e.g., Balb/c, C57/Bl, etc.) have been established, and animals are shared between users to avoid colony duplication and surplus animal production. Additionally, continuous breeding is avoided to prevent the production of surplus animals.

Techniques such as organoids, co-cultures (e.g. PDOX-derived organoids and peripheral blood mononuclear cells (PBMCs) from healthy donors or patients), and ex vivo models (e.g. brain slice) allow for preliminary testing before live animal involvement, further reducing the number of animals required. *For example, from one patient-derived xenograft in a mouse brain, we can extract 5–20 million human tumor cells. These isolated cells can be used to generate uniform organoids for high-throughput ex vivo experiments (1000 cells/organoid). With one mouse, we can generate 5,000–20,000 organoids, allowing simultaneous testing of >1000-2000 drugs. This would never be possible directly with the use of animals. Only the most promising candidates are evaluated in animals at the highest therapeutic dose without toxicity.*

- Refinement - To enhance animal welfare, experimental techniques to reduce distress and improve living conditions are used. For instance, MRI helps not only in reducing animal numbers but also in better assessing disease progression, enabling the humane sacrifice of animals before the onset of symptoms. Other non-invasive techniques, such as oral swabs for genotyping, have been developed, minimizing the need for more invasive procedures. In chronic lung infection models, an oro-tracheal intubation technique has been developed to avoid tracheotomy, thereby reducing pain and discomfort.

The animal facility's full service ensure that animals are handled by highly trained and skilled personnel, reducing stress and potential distress. All personnel working with laboratory animals—including caretakers, researchers, and principal investigators—undergo specialised training. In Luxembourg, a rodent-specific training program has been put in place to ensure that all users, caretakers, and researchers are well-trained to maintain the highest standards of animal welfare. Individuals responsible for performing procedures on animals complete a 40-hour course in

Laboratory Animal Science covering legislation, safety, housing, animal welfare, the Three Rs, and the genetic background of laboratory animals. Personnel who design animal experiments and oversee their implementation undertake an 80-hour course that includes project design and supervision. Continuous guidance and lifelong learning are emphasised to guarantee optimal animal welfare.

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

The category "other" was not reported.

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

No uses of animals took place in categories where a method or testing strategy for obtaining the results sought, not entailing the use of live animals, which are recognised under the legislation of the Union.

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

- **Species: /**
- **Numbers of animals: /**
- **Whether exceeding the 'severe' classification was pre-authorised or not: /**
- **Details of the use: /**
- **Reasons why the 'severe' classification was exceeded:** In 2022 there was no case where the severe classification has been exceeded.

Malta

not provided

The Netherlands

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

In 2023 a total of all uses, excluding GAA creation and maintenance, of 351 872 was registered (versus 2022: 430 692). In 2023 the numbers and uses of animals for the creation and maintenance of genetically altered animals registered was 11,276 (versus 2022: 6 368). Totalling to 363 148 uses in 2023. This sharp decrease of almost 80 thousand uses (-17%) can be explained by the fact that specific large scale projects ended in 2022 and did not return in 2023. Another explanation is that certain economic activities had a lower output in 2023 in comparison to the previous year, resulting in fewer uses within specific fields. A specific increase in the uses for the creation and maintenance of GAA lines can be seen, from 1 509 uses in 2022 to 7 520 uses in 2023.

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

Since there has been a significant decrease in number of uses this translates into every category of main purposes except for “Protection of the natural environment in the interests of the health or welfare of human beings or animals”(+10 418 uses). The main purposes that show a significant decrease in uses are: “Basic Research”(-51 280 uses), “Translational and applied research” (-20 329 uses) and “Regulatory use and Routine production” (-8 795 uses).

The main decrease within the category “Basic research” have been registered in Ethology / Animal Behaviour /Animal Biology (-29 375 uses), Oncology (-16 242 uses) and Immune System (-3 775 uses). The main decrease within the category “Translational and applied research” have been registered in Animal Diseases and Disorders (-9 445 uses), Animal Nutrition (-3 847 uses) and Human Nervous and Mental Disorders 9-2,840 uses). The main decrease within the category “Regulatory use and Routine production” is due to a decrease (-6 556 uses) in Quality control (incl. batch safety and potency testing). The decreases are largely due to specific large multiannual projects ending in 2022 with no continuation in 2023. Also due to economic circumstances a significant lesser amount of quality control batches have been done.

Per species the largest decrease can be seen in the categories:

Species	2022	2023	Difference
Other fish	90 401	68 807	-21 594
Domestic fowl	39 894	22 010	-17 884
Mice	128 201	111 626	-16 575
Zebra fish	18 012	2 757	-15 255
Rats	90 170	81 415	-8 755

Per species the largest increase can be seen in the categories:

Species	2022	2023	Difference
Cattle	2 236	4 215	1 979
Pigs	7 071	8 882	1 811
Sea bass	0	1 029	1 029
Guinea-Pigs	4 233	4 711	478
Other rodents	741	1 088	347

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

The trends in severities is relatively stable, a small change has occurred the amount of *mild* has gone up form 52% to 61%, *moderate* has decreased from 41% to 31%.

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

In the Netherlands, continuous efforts have been taken to promote the principles of the 3R's. However, it is not possible to trace back these efforts to specific items in the statistics.

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

For 2023 two categories need further breakdown, *other birds* (46% or 19 126 uses) and *other fish* (93% or 68 807 uses).

Other birds are mainly: *Parus major* (15.6%), *Cyanistes caeruleus* (8%) and *Anas platyrhynchos* (5%). *Other fish* are mainly *Anguilla anguilla* (72.2%), *Pimephales promelas* (12.1%) and *Gasterosteus aculeatus* (4.5%).

(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 25 records, with a total of 479 uses (2022: 654 uses), have been registered. The species used are: mice, rats and rabbits. As explanation for the use of animal testing 19 entries state that an in vivo test is used as a follow-up of a positive in vitro test. In 6 records the explanation registered is that the test was necessary to fulfil regulatory requirements.

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

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

In 2023 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 the last few years, Poland has seen a slight overall downward trend in the number of animals used in experiments. This is believed to be due to greater awareness among those carrying out experiments (thanks to ongoing training) as well as the work of ethics committees on animal experiments which are uniquely composed when compared to equivalent bodies in other Member States (the committees comprise scientists, humanists and representatives of organisations with the statutory objective of animal protection).

(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 is a consequence of the end of one type of experiment and the start of others, connected to the receipt of research grants linked to an increase in the popularity of a given field of research or orders from external parties.

In 2023, a significant increase was observed in the number of fish used. This is believed to be directly linked to the implementation of the European Commission's recommendations on animal marking, including fish. The number of animals used has been influenced in particular by a scientific project started in 2023 which involved marking sea trout by resection of the adipose fin – 67 000 autumn fry were marked. Once marked, the fish are stocked out to Polish marine areas.

(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 the animals used and not the severity described in experiment approvals, there appears to be a downward trend in the number of animals being used in severe procedures, with an increase in mild and moderate procedures. This trend confirms the possible overestimation of the severity of procedures in experiment approvals. Animal protection organisations are well represented within the Polish competent authorities, i.e. the local ethics committees on animal experiments, and observations show that the representatives of these organisations often propose increasing the severity category.

The significant increase in the number of animals used in mild procedures in 2023 is related to the experiment involving fish marking referred to under point (b).

(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 local ethics committees have a direct impact on the application of the three Rs. 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. In each case, welfare teams also monitor how the three Rs are being applied based on the conditions of the case. To that end, the KKE organises annual in-person training for members of the local ethics committees, as well as monthly online training for both the members of the local ethics committees and for welfare teams. The training covers a wide range of topics.

Moreover, it seeks to tackle current needs and to make new recommendations from the European Commission better understood. The KKE also sets out good practice and its own recommendations, which are communicated directly to those concerned, disseminated on the KKE's website and often discussed during training sessions. These activities improve how the system works, which is reflected in statistical data in subsequent years, in particular in terms of reducing the number of animals used.

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

In 2023, as in previous years, animal species reported in the 'other' field were mainly wild animal species. In Poland, there is a relatively large group of researchers who conduct experiments on these species. For example, in 2023, more than 51% of testing on birds, 91% of testing on fish and 100% of testing on amphibians used wild species.

In Poland, there is also one user which, under procedures required by law as part of routine production, employs tests not included in the list of purposes provided for in the report. Those tests are therefore reported in the 'other' category. These tests concern herbal medicinal products and in 2023, the user's activities accounted for nearly 77% of tests conducted in Poland 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 2023, such cases were identified in six instances. Explanations point to inconsistencies with other legislation or the specific nature of the tested product as the reason for these. The research indicated still requires animals to be used even though validated methods have been developed:

- European Pharmacopoeia (Ph. Eur.) test for pyrogens (General Chapter 2.6.8).
- The ISO 10993-11:2017 group of standards concerns testing of medical devices. 'Biological evaluation of medical devices – Part 11: Tests for systemic toxicity (point 4.1. General, Annex B, Dosage volume, B.1 General, Annex G Information on material-mediated pyrogens)'. PN EN ISO 10993-1: 'Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process' (Annex A Biological evaluation tests). PN EN ISO 10993-2: 'Biological evaluation of medical devices – Part 2: Animal welfare requirements'.
- Research based on OECD Guideline No 203 'Fish, Acute Toxicity Testing', one of the species recommended for testing is rainbow trout (*Oncorhynchus mykiss*).

(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 2023, no such cases were found.

Portugal

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

In 2023, there was a decrease in animal use compared to 2022.
The total number of animals used in 2023 was 86 406, which constitutes a 5,35% decrease in animal use, compared to 2022 (total uses: 91 287).

This figure includes the number of animals used for the creation of genetically altered lines, 3 319, which represent 3,84% of all animal uses. From this total number, 1 282 of these animals were used for the creation of the new genetically altered animal lines for the first time.
The figure also includes the number of 4 224 animals used for the maintenance of genetically altered lines which represent 4,89% of all animal uses.

In the uses of animals for research, testing, routine production and education and training purposes (total uses: 78 863), mice continue to be the most used animal species (56.37%), followed by Zebra fish (18,85%), Sea bass (9,23%), Salmon, trout, chars and graylings (5,45%), other fish category (5,41%) and Rats (4.19%).

There was a decrease in the use of Mammals (total uses: 48 109) and Cephalopods (total uses: 24) and an increase in the use of Fish (total uses: 30 716) and Amphibians (total uses: 14). No Bird was used.

There was a decrease on the reuse of animals for research, testing, routine production and education and training purpose, with a total reuses of 1 608 animals, representing only 2.04% of the total number, compared to the previous year (total reuse of 1 908 animals, representing 2.36% of the total number).

Basic research (61,4 %) continues to be the most frequent purpose for which animals were used to, followed by translational and applied research (34,6%), and by Protection of the natural environment in the interests of the health or welfare of human beings or animals (3%), and Training for the acquisition, maintenance or improvement of vocational skills (0,9%).
The use of animals for Regulatory and Routine production only represents 0,1% of all uses reported, with a slight decrease compared to the previous year.

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

In Translational and applied research there was a significant increase in studies in Non-regulatory toxicology and ecotoxicology (from 0 animals, in 2022, to 720, in 2023) which used Zebra fish.
Studies in Human cancer also represent a significant increase in the use of animals (from 2101, in 2022, to 11 859, in 2023), as well as in Human respiratory disorders (from 6, in 2022, to 355, in 2023).

However, studies in Animal nutrition and in Human infectious disorders have reduced, from 15 423 animals, in 2022, to 9 343, in 2023, and from 4 544, in 2022, to 774, in 2023, respectively.

In Basic research, there was an increase in studies in Oncology (from 6 272 animals used in 2022, to 9 226, in 2023), in Multisystemic (from 5 972 animals used in 2022, to 8 474, in 2023) and in Cardiovascular blood and lymphatic system (from 1 801 animals used in 2022, to 2 265, in 2023).

On the contrary, there was a decrease in the use of animals for studies related to Nervous system (from 17 269 in 2022, to 12 564, in 2023), to Immune system (from 14 364 in 2022, to 12 287 in 2023) and to Ethology/Animal Behaviour/Animal Biology (from 3 211 in 2022, to 1 061 in 2023).

There was also an increase in studies in Protection of the natural environment in the interests of the health or welfare of human beings or animals (from 0 animals in 2022, to 2 393 in 2023), which used Sea bass and Other fish.

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

The trend in the proportions of animal use by actual severity experienced remains the same compared to the previous year, i.e., from the highest to the lowest use, in the categories:

- Mild: 53,21% (2022: 50,11%)
- Moderate: 33,28% (2022- 27,79%)
- Severe: 11,65% (2022: 14,46%)
- Non-recovery: 1,86% (2022: 7,64%)

Nevertheless, there was a decrease in the number of animals experiencing Severe and Non-recovery actual severities and an increase in the number of animals experiencing Mild and Moderate actual severities.

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

There was no particular effort, only the application of the principles of 3Rs when planning and when evaluating projects submitted for authorisation to the competent authority, in addition to those that are applied and considered case-by-case, in each establishment where animals were lodged.

(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 2023 (total uses: 4 315), compared to 2022 (total uses: 14 332).

In the entry of Other fish, and among the several species that were reported of having been used, *Sparus aurata*, *Solea senegalensis*, *Argyrosomus regius* and *Atherina presbyte* were the most used animals species, representing, respectively, 44.50%, 25.75%, 9.46% and .5.79% of all uses of this entry.

There was a decrease in the use of Other mammals (total uses: 26), compared to the previous year (50), belonging to studies of Ethology/Animal Behaviour/Animal Biology with the following animal species:

- *Crocidura russula*
- *Globicephala macrorhynchus*

Nevertheless, there was an increase in the use of *Crocidura russula* compared to the previous year, as in 2023 there were studies using this small insectivorous mammal.

(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:

19,065 animals were used in procedures in 2023 which was 13.73% more than in 2022 (16 446 animals). The species most used in procedures were mice (whose number increased from 6 238 in 2022 to 7 657 in 2023), rats, domestic fowls and guinea pigs.

(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 zebra fish used in procedures increased by 100% in comparison with the previous year. The procedures were performed for translational and applied research (human nervous and mental disorders).

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

The proportion of tests carried out under general anaesthesia and from which the animal did not regain consciousness was lower than in 2022 as a result of a better assessment of the severity of procedures.

The percentage of uses classified as moderate and severe decreased in favour of the mild uses due to efforts made by animal users to reduce the severity of procedures through the implementation of refinements and earlier humane endpoints.

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

Involvement of the National committee for the protection of animals used in scientific purposes for better understanding by the users of the legislative requirements.

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

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(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:

A number of 18 rabbits were used for pyrogenicity testing within regulatory use/ quality control; the tests were performed for countries where alternative test is not an official method and where the Russian Pharmacopoeia is used. Pyrogen testing is a statutory requirement of the Russian Pharmacopoeia to ensure product safety.

(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:**

Slovakia

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

Overall, there is a trend towards fewer animals being utilised in research, with 2023 seeing the lowest number. A small number of species, meanwhile, showed an increase. Because of the nature of the research, more rats and rabbits were used. In particular, more studies in the fields of endocrinology, cardiovascular disease, and reproduction were carried out using these species as the preferred model. Additionally, we report on the use of sheep once more after a three-year gap. These 35 animals come from one establishment focusing on gastrointestinal research that was being finalized this year.

(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 used in regulatory testing has decreased. This could be explained by the animal reduction initiatives mentioned in section (d). On the other hand, basic research increased as a result of the type of the projects approved and carried out. This year, no ethology or animal behaviour studies were recorded, owing to a lack of use of Japanese quails, which contributed substantially to this category in previous years. This year, significantly more animals were used in endocrinology studies. The increase in trend is most likely attributed to renewed interest post-Covid 19 pandemic and energy crisis, which hindered substantial research during those period in this topic. Furthermore, this year we have seen a considerable increase in the use of animals in multisystem research. Upon further examination, we can conclude that the rise in the topic is attributable to advances in the nanotechnology research sector, as well as the application of unique methodologies using nanoparticles by various research teams/establishments in Slovakia. We predict the trend to continual increase in the coming years. On the other hand, fewer animals were utilised in studies related to the immunological and respiratory systems. The decline in research funds focussing on COVID and related illnesses in the preceding years (2020–2022) may be linked to this.

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

We report a nearly 50% decrease in severe severity this year compared to the prior reporting year, but an increase in the moderate severity category. Following RA, a large number of projects that had an overall severity classification of severe were reclassified as moderate. The efforts made to improve toxicity research, as mentioned in section (d), could be contributing factor.

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

The educational courses that State Veterinary and Food Administration of the Slovak Republic (SVFA) promotes 3Rs principles, with special efforts to improve the welfare of laboratory animals in research where the potential for pain and suffering is largest. Majority of such research comes under regulatory toxicology studies. As a result of common effort, most of the research groups conducting regulatory studies, whenever possible, avoid using higher dose of chemical/ substance and rather look for clinical signs that point to possible severe toxicity and death if higher concentration would be given. This approach effectively refines and reduces the number of animals and suffering in toxicology research. In addition, one research establishment working in partnership with the Faculty of Informatics and Information Technologies aims to decrease the use of laboratory animals in medical research by using artificial intelligence to create models for

predicting a substance's toxicity. As a refinement to standard protocols, we are currently working to encourage the use of micro sampling in place of routine blood collection.

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

Every year, we report on the use of birds (other) in this category. These animals came from a single facility that specialises in neurological research. The total number of these birds used was approximately half that of the previous year. One of the reasons is that the establishment did not use Japanese quails this year, which normally account for more than half of all other birds 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:

In order to assess the study hypothesis or set of objectives, researchers are strongly encouraged to adopt validated procedures that replace the use of animals. Project proposals that use live animals in spite of the availability of alternate methods are therefore rejected.

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

There were no such cases in 2023 when the severe classification was exceeded.

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

Slovenia

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

In 2023, 4 735 animals were used in research in Slovenia, compared to 2022 when 6 523 animals were used. In the largest numbers, laboratory rodents were used. The most used animal are mice (80% - 2023 and 77% - 2022). Comparing the numbers of the last 5 years, the number of animals used in research in Slovenia has been decreasing over the years.

As in previous years, the largest number of animals was used for the purpose of translational and applied research (44,1%). This is followed by regulatory use and routine production for the purpose of the pharmaceutical industry (36%), basic research (15,3%) and, to a lesser extent, higher education and the maintenance of professional competence and the protection of the natural environment to protect the health or welfare of humans or animals. Most animals are used in research on cancer, digestive tract, and immune and endocrine systems. A large proportion of animals are also used in the pharmaceutical industry for quality control, including the safety and efficacy of medicines.

Number of authorised projects was higher in 2023 than in the previous year, however the number of animals used in 2023 is lower.

Cats and dogs are not used in research in Slovenia. We also do not record the breeding or use of non-human primates.

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

Compared to previous year, there is a decrease in the field of re-use. The proportion of animals re-used dropped from approx. 22% to 10,6%. The significant decrease of the re-used animals was in mice for regulatory use and routine production where the number is more than 50% lower. The reason for that is that the absolute number of mice has dropped because of lower batch number that needed to be tested. Number of batches varies based on the demand on the market and production capacity.

In 2023, 4 627 not genetically modified animals were used, which is almost 98% of all animals used. The remaining animals were genetically modified mice, all without an expressed harmful phenotype, compared to previous year where 18% of genetically modified mice was with an expressed harmful phenotype. These mice were used as part of basic research for the purpose of nervous system studies.

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

As in previous years, most procedures in Slovenia (78.3%) are classified as “mild” in 2023. In procedures classified as “non-recovery”, less than 2% of animals (mice) were used. In “moderate” procedures less than 20% of animals were used, mainly mice, rabbits, and rats. As “severe” procedures are concerned, less than 1% of animals (mice) were used in 2023. The number is slightly higher than in previous year. In 2022, 29 mice and in 2023, 37 mice were used in studies of the nervous system, infectious disorders in humans, non-regulatory toxicology and ecotoxicology, and protection of the natural environment to protect the health or welfare of

humans or animals. This slight increase in numbers can be attributed to the type of the project and more precise use of scoring sheets for the assessment of animals.

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

Reduction, replacement, and refinement principle are promoted through education and training courses for persons working with laboratory animals (some e-modules are already included), regular meetings with animal welfare officers, members of ethical committee, promoting the use of ETPLAS and e-modules, maintaining the competence (form with updated information on trainings, workshops, conferences, etc. must be attached to the project application file), etc.

In 2023, new ethical committee was appointed for a period of four years. One of the selection criteria for new members was also completed e-modules at ETPLAS, at least EU-12 – severity of procedures and EU-25 - evaluation of projects. Ethical committee checks 3R principles during each evaluation process of projects. Beside the evaluation, they give advice to users to promote the use of 3R principle.

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

Nothing was reported under category "other".

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

Regulatory organs still require performing rabbit pyrogen test when there is LER (low endotoxins recovery) effect in BET method suspected or confirmed, to show if the rabbits can detect masked endotoxins. It is also required in registration process to show absence of pyrogenic substances with non-endotoxin origin. In-vivo testing of endotoxins/pyrogenic substances (like rabbit pyrogenicity testing) represents very low number of all tests (in-vitro and in-vivo) that detect endotoxins. There is ongoing plan to implement MAT test.

Rabbits are still used in one pharmaceutical company where they have been intensively dealing with the replacement of pyrogen test with MAT test on monocytes since mid-2023. They plan to renovate the laboratory, so that it will suits the MAT test, furthermore, to qualify the equipment, transfer of the method into the laboratory, validation of the method. The implementation of the MAT test is foreseen for the end of next year.

In the meantime, they apply refinement procedures, e.g., re-use of animals, gradual adaptation of animals to the handling and procedure, rehoming after the end of use.

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

- **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:**

“Severe” classification was not exceeded.

Spain

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

In 2023, the number of uses of animals for scientific and teaching purposes increased by 20 969 compared to 2022. This increase is due to the higher number of authorised projects in 2023, 12.5% more than in the previous year.

The uses of most animal species have not undergone significant changes, but there are some exceptions.

In 2023, no procedures were carried out on turkeys or rhesus macaques. Both are species that have been used in very low numbers in previous years. Conversely, less common species such as the Chinese hamster or fish of the poeciliidae family have both been used in projects in 2023.

The number of uses involving other species has undergone large percentage changes, although given that the numbers of procedures are not very high, relatively small changes mean, in percentage terms, large differences. This is the case for uses involving reptiles, used in seven projects (in 2022, two more were developed and have now been completed), most of which focus on the study of ethology, biology and animal behaviour; ferrets, used in three projects for the study of the nervous system (in 2022 there were five projects) and xenopus (used in basic research projects, mainly with the objective of gaining a deeper understanding of the developmental biology or in oncology studies).

In the case of crab-eating macaque, their use has been reduced to less than half due to a specific health problem in one of the centres with the most research activity, leading to a temporary halt in research using primates.

The largest increase, both in terms of percentage and absolute number of uses, occurred in the sea bass species, again linked to the need to increase understanding of fish species that can be bred for agricultural purposes. The uses, almost 97% of which are 'up to mild' severity) are intended, in 95% of cases, to increase understanding of the nutrition of these animals.

Uses of octopuses have increased, in order to increase knowledge of their nutritional mechanisms, diseases that may affect them, their prophylaxis and above all, to understand and identify factors that affect their well-being, in particular by looking for non-invasive indicators thereof.

Changes in uses of ‘other species’ are addressed in section *(e)Further breakdown on the use of ‘other’ categories if a significant proportion of animal use is reported under this category*

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

Although the total number of uses of animals has not changed significantly, there are notable changes in some areas:

In translational and applied research, the number of procedures increased by 10%, due to the fact that uses for research into animal nutrition have multiplied by 2.5. In parallel, compared to 2022, there has been a 10% decrease in uses relating to animal welfare research. Both changes are due to the uses carried out at very early stages of the development of farmed fish.

In 2023, there was a large percentage change in animal uses intended to maintain colonies of genetically modified animal lines, as it was halved compared to the reported number of animals used in 2022, due to the decrease in activity at one of the centres and the implementation of non-invasive tissue sampling techniques, among other reasons.

Use in higher education has led to a 27% increase in the number of uses, due to the rise of specific training courses, mainly in microsurgery.

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

There has been a significant change in the proportion of severities, with an increase from 49% to 66% in uses classified as 'up to mild' and a decrease in procedures classified as 'moderate' from 42% to 26%. 'Non-recovery' uses remain stable compared to 2022 and 'severe' procedures decreased by 1% (around 10 000 fewer 'severe' uses than in 2022).

This change from the previous year is mostly due to uses involving fish with productive capacity, relating mainly to research into their nutrition and, to a lesser extent, their welfare.

Given that the initial stages in the development of fish bred in captivity are the most complicated, uses are often carried out in the early stages of life with populations which, although high in terms of the number of individual fish, often have a very small number of lays. Thus, a small change in the number of projects causes the number of animals involved to vary greatly. In 2023, 'mild' procedures increased (both in absolute and percentage terms), while 'moderate' and 'severe' procedures decreased because fewer projects involving temporary deprivation of food were carried out.

In general, and not solely relating to uses involving fish with productive capacity, better implementation of monitoring protocols and the establishment of endpoints more appropriate to the types of procedures and animals used have also contributed to the reduction of procedure severity.

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

General Measures

- Training of staff involved, including inspectors. Some regions have established pre-training with simulators and *ex vivo* models before any procedures can be carried out on animals.
- Appropriate activity records that enable the identification of situations in need of improvement and lines of action to address these improvements.
- Dissemination of information on the three Rs principle (to researchers, animal welfare, project managers and others involved), including via mail delivery, thematic meetings and use of websites.
- Inspections of the centres in order to strengthen the application of alternative strategies, especially as this affects refinement (indication of corrective measures both for facilities - material, dimensions, flows, biodiversity measures, environmental enrichment elements, etc. and in the various control records (animals, staff, material, etc.))
- Audit the process of document searches for the application alternatives for each project that is authorised, as well as a follow-up of the documentation provided.
- Strengthening the work of animal welfare bodies.

- Improvement or replacement of certain techniques, such as replacing genotyping with tail cutting or refining it by using local anaesthetic.

Replacement

- Encouraging the use of cell cultures or in vitro, simulators and databases.
- Encouraging the use of audio-visual media, biomodels, cadavers, organs or butcher's or fishmonger's cuts in teaching, avoiding the euthanasia of animals specifically for teaching practices, and as a preliminary step to using live animals.
- Promotion and verification of the use of preliminary in vitro tests where possible.
- Carrying out preliminary in vitro studies in research projects to determine their validity prior to using animals.

Reduction

- Encouraging the sharing of organs and tissues of euthanised animals.
- Improvement in the management of animals not used in every project.
- Improvement of accurate statistical analyses to minimise the number of animals used,
- Cryopreservation of genetic material.
- Encouraging the sharing of organs and tissues of euthanised animals.
- Encouraging the use of pilot studies that can help, for example, to select the correct group sample size;
- Reducing the sources of variation: procedural conditions, animal conditions (sex in particular) and environmental conditions.
- Sequencing of procedures.
- Sharing control animals, especially in the manufacturing of vaccine batches.
- Use of relevant technology (e.g. multiphoton microscope allowing for intravital microscopy, thereby reducing the number of animals needed to obtain statistically relevant data, or resonance imaging to obtain data throughout the monitoring period, i.e. not requiring the euthanasia of animals to obtain results at different times).

Refinement

- Comprehensive attention to housing conditions of the animals, including how they adapt to them.
- Specific monitoring of post-operative facilities for animals: e.g. providing thermal blankets for rodents
- Monitoring protocols specific to the circumstances of each procedure, with expected parameters according to the type of procedure or animal species and including corrective measures and end-point criteria.
- monitoring the anaesthetic and analgesic techniques involved in procedures;

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

In general, the number of procedures on 'other species' is not very high, with the exception of 'other fish' and therefore any changes in the small number of procedures seem, in relative terms, very significant. These are often projects developed using wild animals, with severity classification 'up to mild' and with subsequent release into the wild. The most common objectives are the protection of the environment in the interest of the health or welfare of humans or animals, the preservation of species and a greater understanding of the species itself.

- Other rodents

The number of procedures has increased significantly (from 94 uses in 2022 to 695 in 2023 (i.e from 0.02% of rodent uses to 0.14%)

The species with the highest proportion of uses is the *Apodemus silvaticu*, whose number of uses have increased from 29 in 2022 to 241 in 2023, mainly for the study of the effects of changes in land use on wild fauna, or the role of wild fauna in reforestation.

Crodiura Russula, *Microtus arvalis* and *Mustela nivalis* are being used for the first time with the objective of improving environmental protection.

- Other carnivores

This year, four wolves and two brown bears were used for the first time (none used in 2022). Both groups were used for species preservation, in a project aimed at improving the detection and quantification of the impacts of wind power development on biodiversity.

- Other mammals

The number of procedures on other mammals has more than tripled compared to 2022.

In almost all cases the objectives are the protection of the natural environment in the interest of human or animal health and welfare (various species of bats and cetaceans), or basic research, in order to better understand the respective species (various cervids, cetaceans and bats). In the case of a project using camelids, the objective is to obtain a method of treatment for humans that prevents infection with the West Nile virus and which could be the basis for treatment against this virus, especially in serious cases of encephalitis in humans.

- Other amphibians

The number of uses involving 'other amphibians' has remained almost the same as in 2022, when the most commonly used ones were:

Pelobates cultripes used in three projects that have been under development for several years, focusing on the developmental biology of the species.

Discoglossus galganoi (16.23% of uses) for the development of impact models on environmental risk assessment and to analyse the impacts of chemicals on amphibian populations, *inter alia*.

- Other birds

The proportion of uses remains the same as in 2022 at around 5% of bird uses, although in absolute terms the number of procedures has decreased.

The number of uses involving *sturnus unicolor* is relatively high in studies on the starling's biology and on its role as a seed disperser in deforested areas of anthropic origin.

The uses involving *taeniopygia guttata* were carried out in a single project that aimed to study environmentally induced epigenetic modifications to the evolution of pigmentation phenotype in birds.

The uses involving *passer domesticus* focus on the studies of ecosystems and the impact of consumption of certain substances on these ecosystems.

In other cases, such as uses involving *columba livea*, uses relate to the study of effectiveness, safety and innocuity of the vaccines for which it is the target species.

- Other fish

Most procedures on 'other fish' were carried out by users engaged in research into marine species of future commercial interest and fish production for human consumption. A number of studies were carried out regarding physiology, immunity, reproduction, feeding and health (study of infectious diseases and their prophylaxis and treatment) and adaptation to the production technologies of these species. The is the case for procedures on sea bream and tuna for example.

In addition to the uses related to productive capacity, these fish have also been used in other areas such as establishing the causality between the reproductive biology of marine organisms and how this is altered by external factors in the context of climate change.

- Other basic research:

Among the basic purposes of the uses reported as 'other', notable examples are uses of animals in palatability studies of products of veterinary use (using dogs and cats), in studies related to acute and chronic pain and options to alleviate it, genetic diseases, gene therapy and studies related to the Micro-Ecological Life Support System Alternative (MELiSSA) project, and microplastics and associated contaminants.

(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:

- Test for pyrogens in rabbits: As part of the quality check on the finished product, with the determination of all pyrogenic agents (not only endotoxins), and included in the various applicable pharmacopoeias, as well as the different marketing licences in force. 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 is only viable assuming the risk of potential pyrogenic substances not due to endotoxins. Following the strategy proposed by the European Directorate for the Quality of Medicines Healthcare (EDQM) of the Council of Europe for the replacement of the pyrogen test on rabbits, the company will develop and validate the Monocyte Activation Test (MAT) in those products for which the pyrogen test is currently required in the monographs of the European Pharmacopoeia. While the MAT has not been definitively developed and validated, the company maintains the test on rabbits as a safety criterion in the administration of blood products to patients and also when required by regulatory bodies and compendial references other than the European Pharmacopoeia and/or provided in the marketing licences.

- Regulatory use/Toxicity and safety. Skin corrosion/irritation in rabbits: Use for extracts, solid materials (tissue, silicone sheets, etc.) of medical devices and to be able to assess their biocompatibility (*in vitro* methods are validated for pure chemicals), the test is performed according to the method described in ISO 10993-10 (Biological evaluation of medical devices.

Part 10: Skin irritation and sensitisation testing). There are alternative in vitro tests that are used at preliminary stages, but there are currently no methods validated by the regulatory agencies to replace this test.

Production of monoclonal Ac by the ascitis method in mice. Use has been made of the animal model at certain times, given the impossibility of meeting production demand due to the non-functionality of the antibodies obtained on the in vitro platforms and the poor performance of those platforms. In parallel, in vitro production continues to be optimised.

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

- **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**

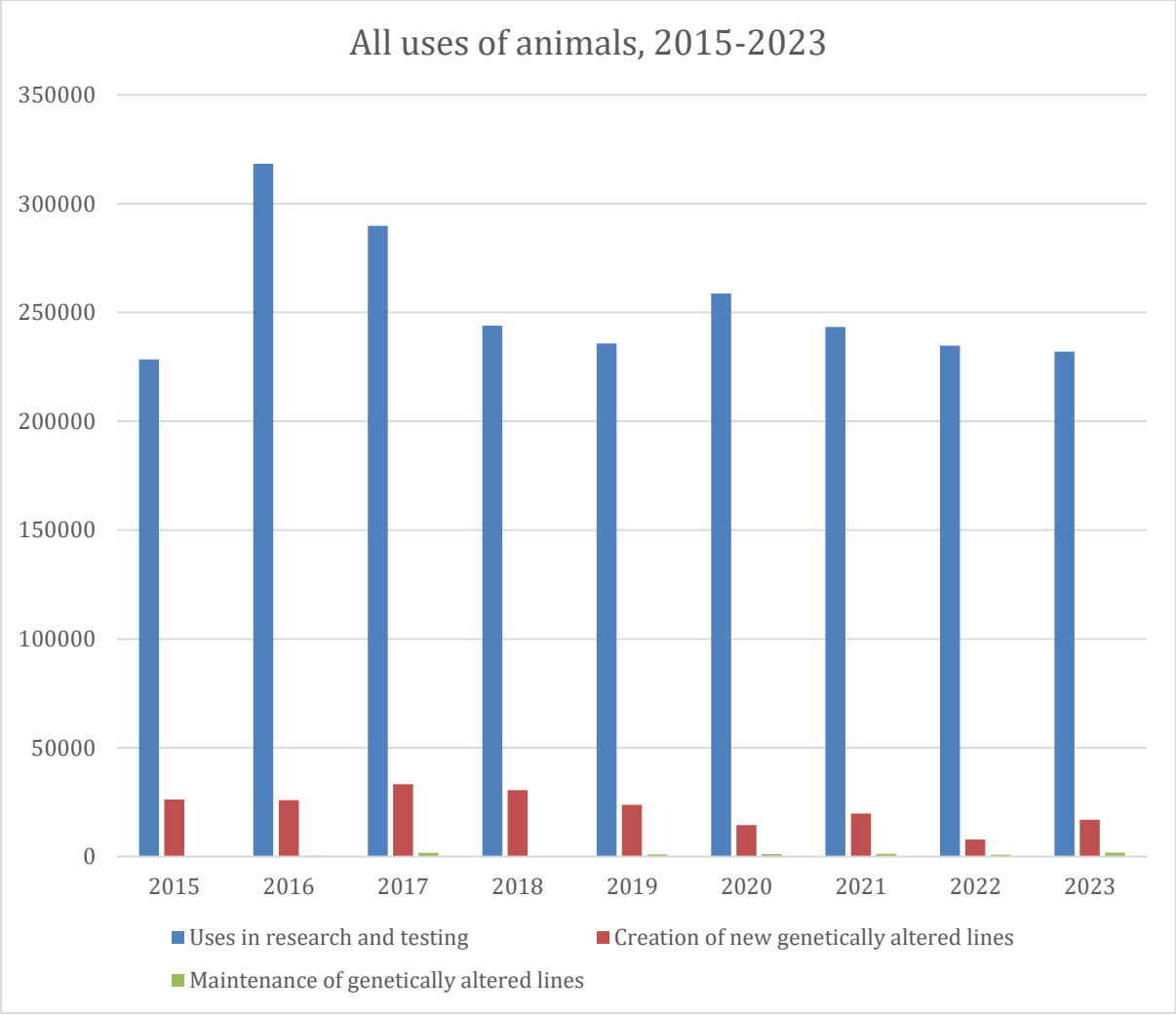
No such circumstances have been given.

Sweden

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

Total number of uses

The total number of uses (re-use and creation, as well as maintenance, of new genetically altered lines included) reported for 2023 (250 686) is similar to 2022 (243 489) and 2021 (264 301). There are only minor fluctuations in the figures over the years since 2015.



No changes in trends have been observed in the proportions of for example *Genetic status*, *Re-use*, *Proportions of animals other than non-human primates by place of birth*, or *Origin of non-human primates used for the first time.*

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

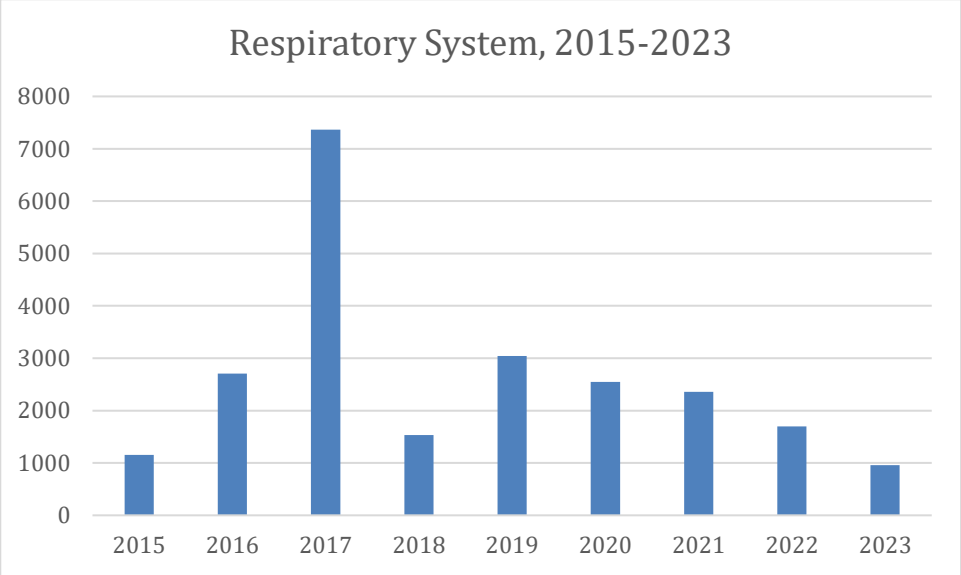
We have selected some interesting areas where we have seen either clear trend differences in uses throughout the years 2015-2023, or a large difference between the uses in 2023 compared to 2022.

Basic research

Respiratory system

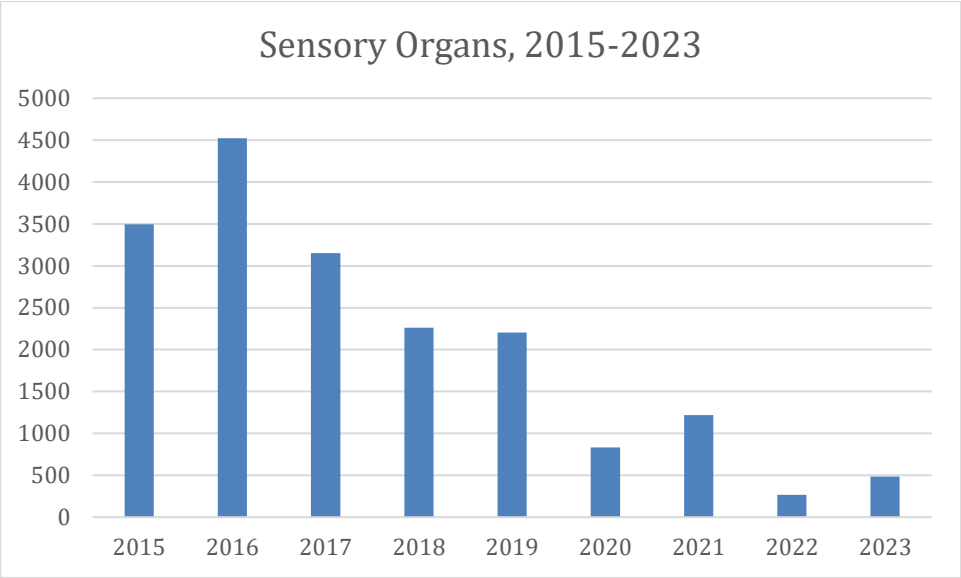
Since 2019 there has been a steady decrease in the number of uses in this category and the number for 2023 is the lowest of all reporting years. The high number in 2017 is partly explained by a large report from a single user. The decrease in uses from 2021 (2 361) to both 2022 (1 695) and 2023 (960) is mainly due to, that in 2021, a single user performed many studies on Covid-19 in combination with having many PhD-students. The number of uses from that specific user has decreased since then. Another interesting observation is that the same user has started using proportionally more guinea pigs than mice, which according to the user will continue since there has been a shift in focus.

Interestingly, in 2023 no rabbits have been reported in this category which is the first time since 2018. This is due to cessation of activity by one user.



Sensory Organs (skin, eyes and ears)

In this category, a clear decrease is observed from 2016 and forward, both in the number of uses and in the number of species used. From 2021 and forward, only mice, rats and guinea pigs have been used. Earlier years, also uses of for example rabbits, zebrafish, domestic fowl and pigs have been reported. The continuous decline in total uses is partly a consequence of the fact that a large user has retired and has started to downsize their activity.

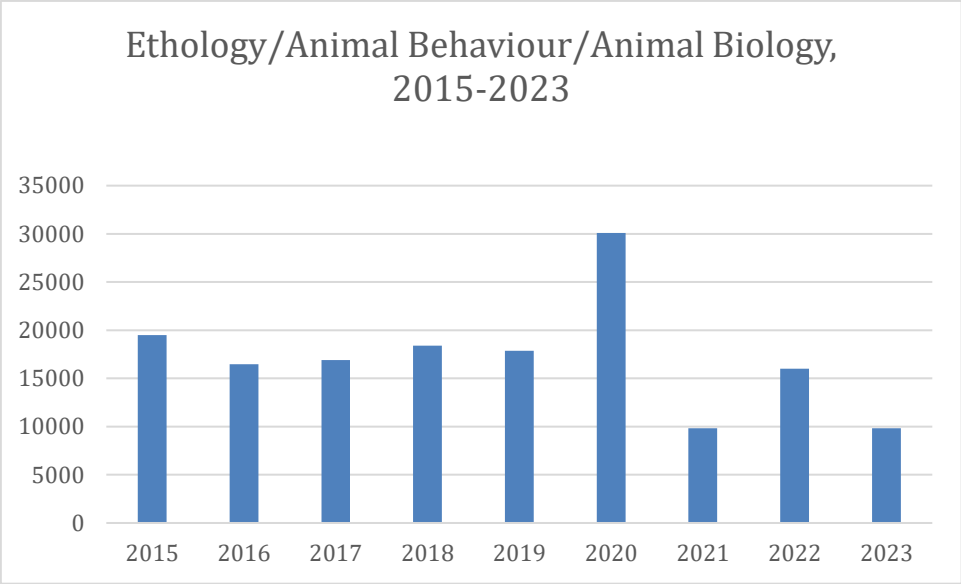


Multisystemic

A large decrease in the *Multisystemic* category was observed in 2023 compared to 2022, from 12 448 to 4 497. This is partly due to the cessation of activity by a zebrafish user, resulting in a large decrease in zebrafish use, from 10 317 in 2022 to 499 in 2023.

Ethology/Animal Behaviour/Animal Biology

In 2023, 9 842 uses were reported in this category. This is a decrease from 2022 with 16 014 reported uses, but very similar to 2021 with 9 838 reported uses. In 2022, the single largest use was 5 000 Atlantic mackerel (*Scomber scombrus*) from one user. In previous years, the uses have been between 15 000 and 20 000, apart from in 2020 when an unusual high amount of salmon use (18 400) was reported from one user.



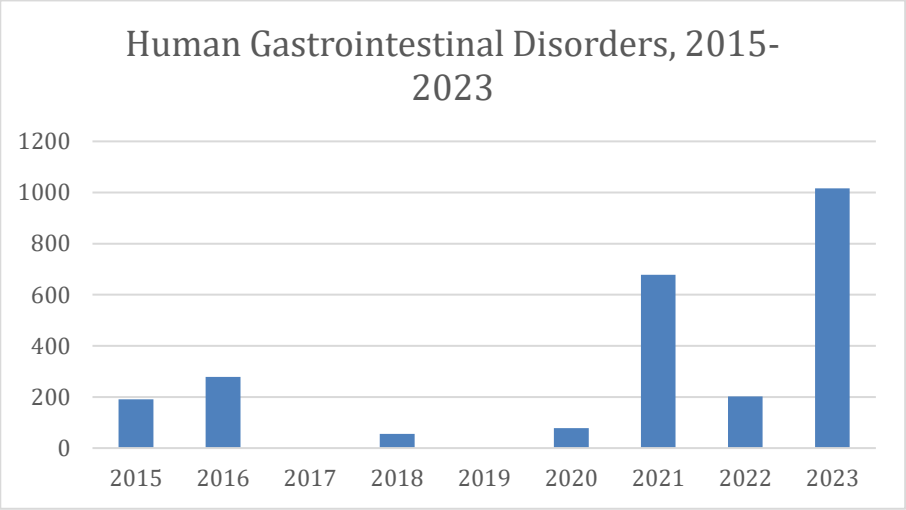
Other Basic Research

The proportion of uses in the category *Other Basic Research* has decreased over the years with temporary minor increases. The decrease is most likely a consequence of the more detailed division into different purposes, which enables more uses to be reported in an accurate category. The total use in category *Other basic research* was less than 1 % in 2023 compared to 4 % in 2022, with a mean of 4 % in the years 2015-2023.

Translational and applied research

Human Gastrointestinal Disorders including Liver

An increase of animal use in *Human Gastrointestinal Disorders including Liver* is observed in 2023 compared to previous years. Between 2023 and 2022, the increase is due to one specific user reporting more than 700 additional uses. However, in order to confirm a trend, more data is needed. The total use in the category was 1 016 in 2023 compared to 202 uses in 2022.



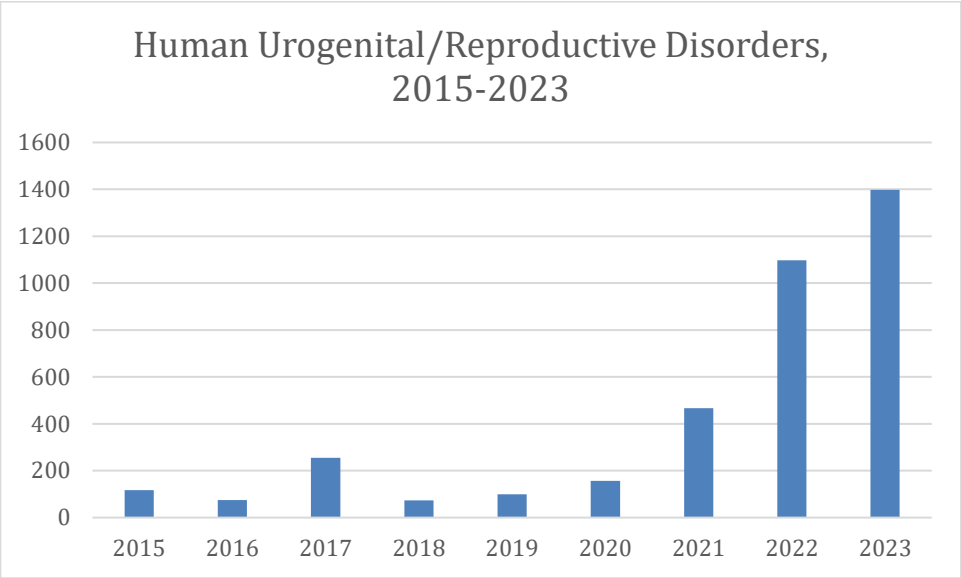
Human Immune Disorders

In 2023, 1 068 uses were reported in this category. This is a decrease compared to 2022 (2 111) but similar to 2021 (1 049). Initially, 2 543 uses were reported for 2022, but when working with this analysis, we found a duplicated report (400 mice and 32 rats), leading to the correct number of 2 111 for 2022. Due to many different users reporting during these years, it is difficult to identify any specific reasons to the changes.

Human Urogenital/Reproductive Disorders

From 2020 forward, there has been a significant increase in number of uses in this category. This is entirely due to one user who started reporting animals in this category in 2021 with increasing numbers each year. Between 2022 and 2023, the numbers have increased from

1 098 to 1 398 uses. However, the biggest increase was seen between 2021 and 2022 from 467 to 1 098 uses.

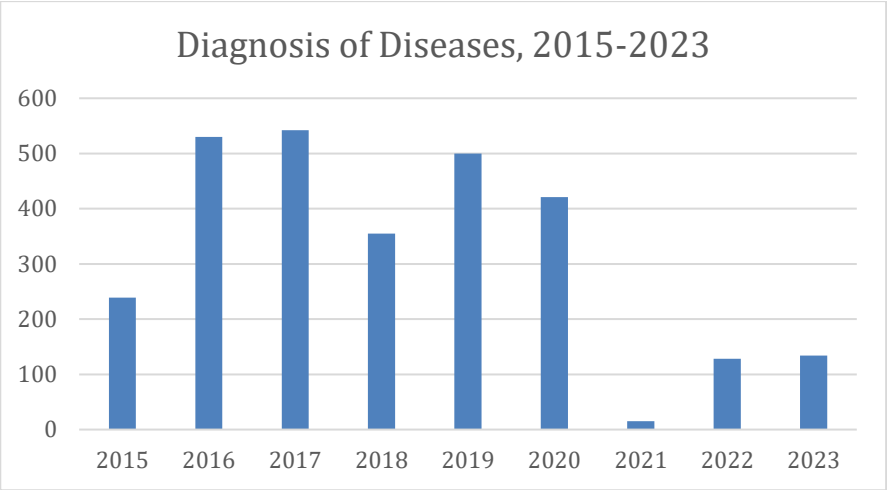


Human Sensory Organ Disorders (skin, eyes and ears)
63 uses were reported in this category during 2023. This is a decrease compared to 2021 (1 199) and 2022 (124). The high number in 2021 is due to a single user reporting 1 054 uses. This user did not report any uses in 2022 or 2023.

Animal Nutrition
This category had a big increase in uses during 2023 (1 434) compared to 2022 (116) and 2021 (806). The increase between 2022 and 2023 is due to a single research group reporting 1 380 uses of salmon, trout, chars and graylings.

Animal Welfare
In 2023, this category had a large increase (2 170) compared to both 2022 (371) and 2021 (267). The main reason for this is two studies on pigs (1 800 uses) where enrichment has been experimented with in order to reduce stereotypical behaviours.

Diagnosis of diseases
Notably fewer uses have been reported in this category in the last three years compared to earlier years. After contact with users, we have reason to believe that this category has been used incorrectly, which could explain the high numbers in previous years.

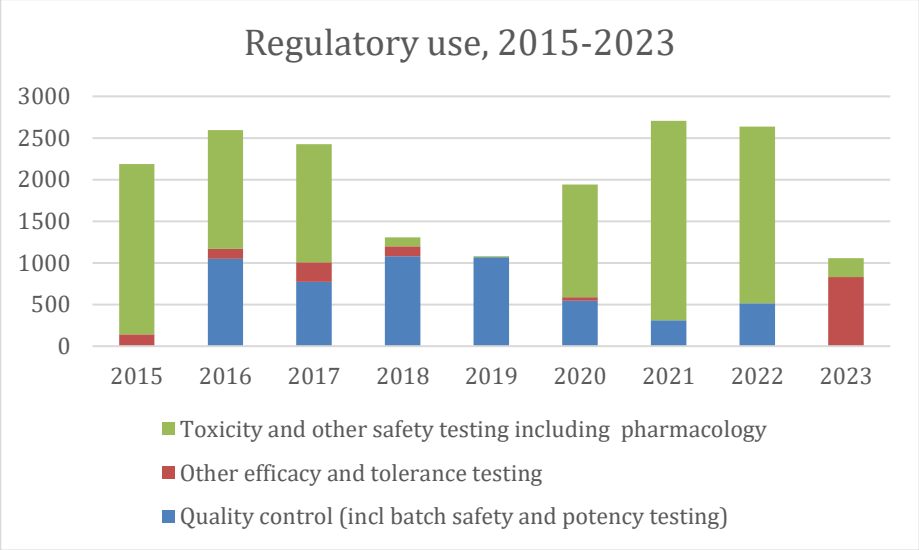


Preservation of species

An increase of animal use in *Preservation of species* was observed in 2023 (10 890) compared to 2022 (1 949) and 2021 (221). This is an effect of one user reporting more than 9 000 uses in 2023, both due to new projects with a different focus than before, as well as a reassessment of the suffering of the animals leading to the experiments being classified as EU-statistics.

Regulatory use

There is a decrease in 2023 (1 056) compared to 2022 (2 636) and 2021 (2 706). However, as Sweden in general has few uses for regulatory purposes, a single study can influence the total number significantly. To determine if this is a general downward trend, more data is needed.



Maintenance of genetically altered lines

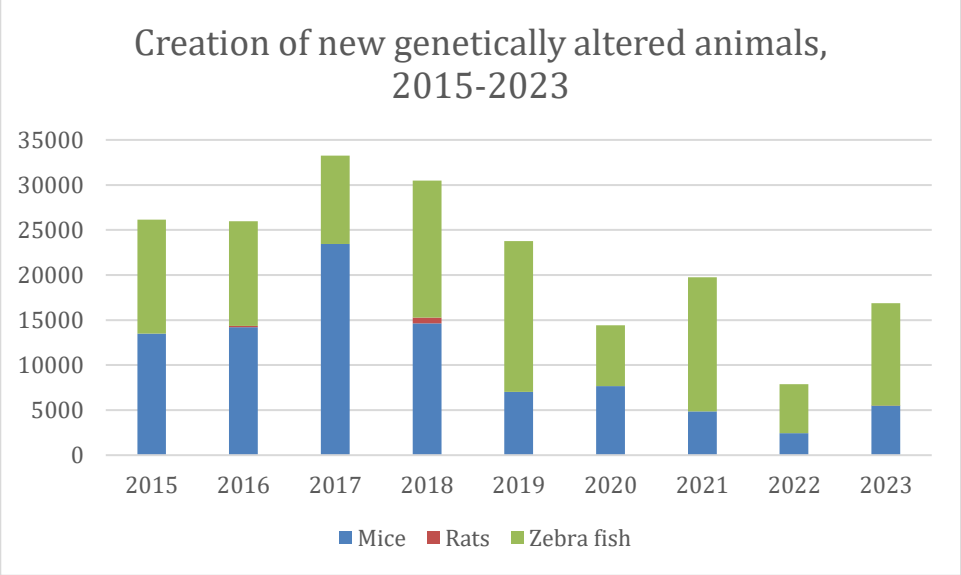
In 2023, there is a clear increase in uses (1 865) compared to 2022 (860) and 2021 (1 225) This is due to a single user reporting 1 754 uses.

Creation of new genetically altered animals

Only three species are used for the creation of new genetically altered animals; mice, rats and zebrafish. There is no clear change in trend in uses over the past four years, however, a drop is observed during 2022. The drop regarding zebrafish uses in 2022 (5 407) compared to 2021 (14 864) and 2023 (11 361) could be due to the move of a big user between facilities.

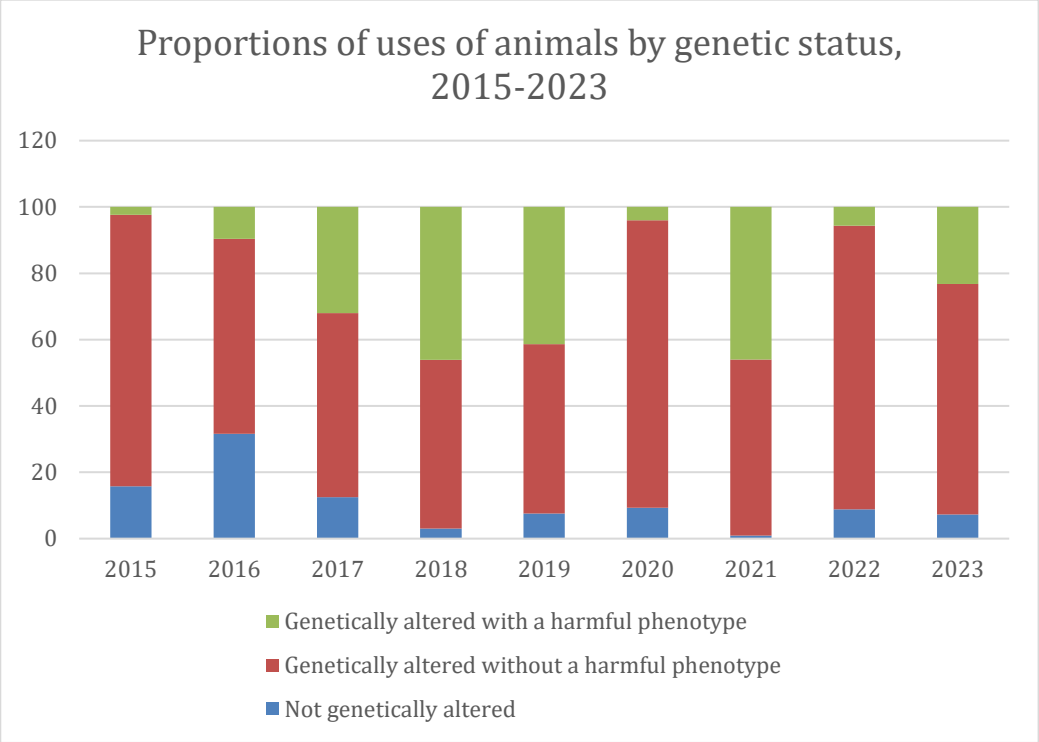
On a longer term, a general decrease is observed in the use of mice. This could possibly be due to a transition from successful creation to maintenance of the genetically altered lines, but in order to determine this, more information is needed.

Very few or no rats are used within this category.



The proportions of uses of animals by genetic status, creation

In general, the proportions are similar over the years with the majority being *Genetically altered without harmful phenotype*, followed by *Genetically altered with harmful phenotype* and *Not genetically altered*. In some years, the proportion of uses of animals with harmful phenotypes increases (2017, 2018, 2019 and 2021). These increases are all due to single users using a large number of animals in the respective years.



Species

Goats
There is a decrease in the uses of goats in 2023 (24) compared to 2022 (45) and 2021 (51). To determine if this is a trend or not, more data from the coming years is needed. The differences can so far be explained by different projects during the different years. For example, 22 uses of goats were reported in an Animal Welfare project in 2022.

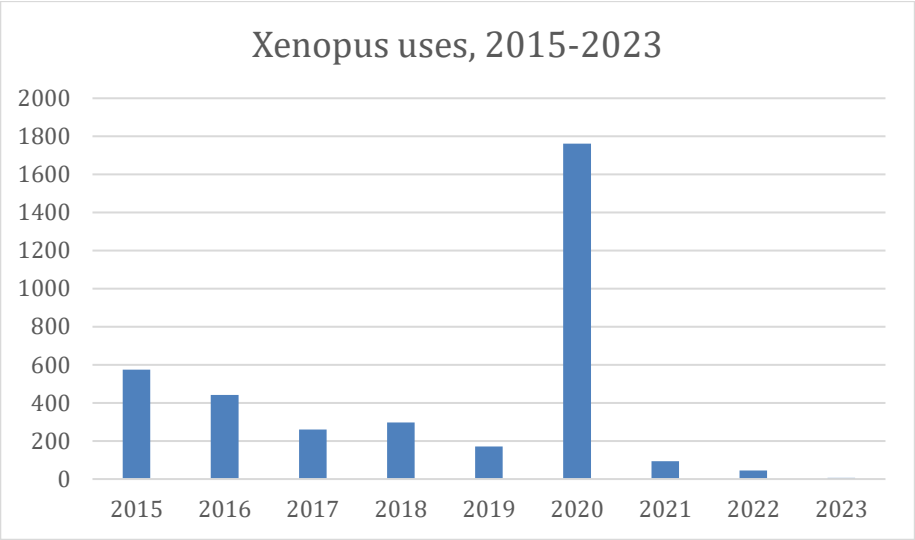
Cattle
The number of cattle used has decreased between 2023 (502) and 2022 (808). The uses of cattle had a big peak in 2021 (3 838). This big peak is mainly due to 3 different users together accounting for more than 3 500 animals. For example, one big project focused on the investigation of *Mycoplasma bovis* infection. This project started reporting animals in 2020 and finished in 2022.

Pigs
There was an almost doubled increase in the number of pigs used from 963 in 2022 to 2 767 in 2023. The increase is mainly due to two studies where enrichment has been experimented with in order to reduce stereotypical behaviours.

Other rodents
In 2023, the use of other rodents is higher (1 085) than in both 2022 (0) and in 2021 (56). The rise is due to one single user reporting 1 805 uses in 2023, both due to new projects with a different focus than before as well as a reassessment of the suffering of the animals leading to the experiments being classified as EU-statistics. All uses in 2023 were within *Preservation of species*. The 56 uses in 2021 were reported from another user, all in *Basic Research/Immune System.*

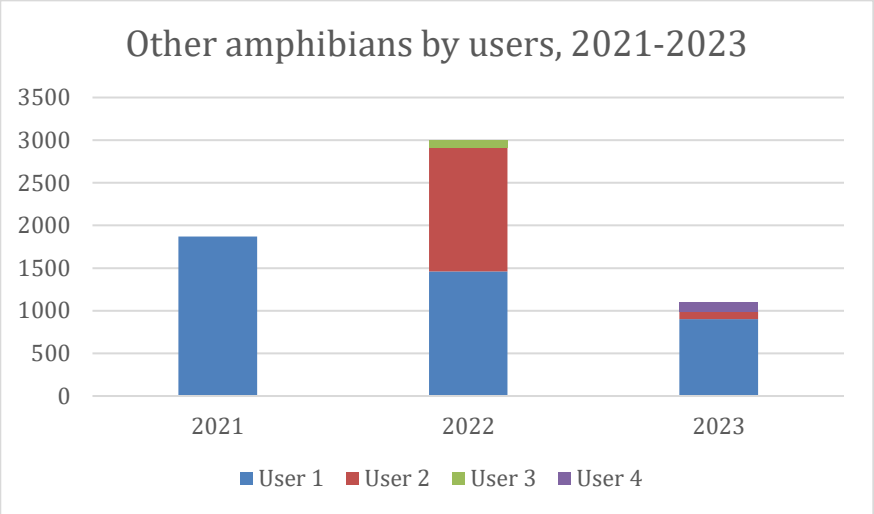
Turkey
Zero uses of turkey were reported in 2023, compared to 2022 when 124 uses were reported from a single user for the purpose of *Translational/applied research, Animal Welfare*. The experiment concerned testing a new stunning method at slaughter.

Xenopus
There are only six uses of Xenopus in 2023, following a decreasing trend. The sharp increase in uses in 2020 can be explained in part by a large study on environmental toxins under the purpose *Protection of the natural environment in the interests of the health or welfare of human beings or animals*. Concerning the decrease, we have received information from one user that they have started importing Xenopus oocytes instead of keeping animals for oocytes.



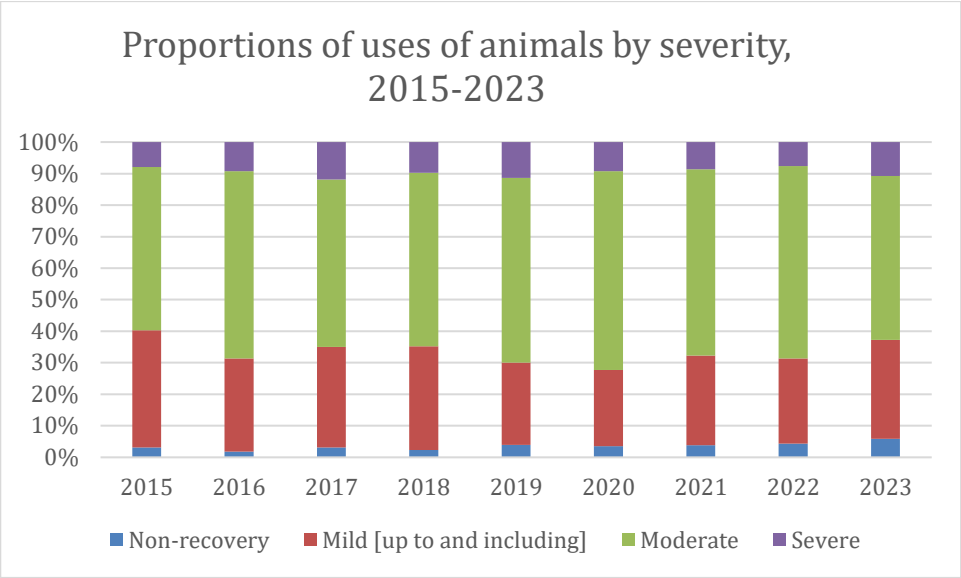
Other amphibians

In 2023, there is a decrease in the use of other amphibians compared to 2022, whereas the 2022 use is higher than in 2021. The rise in 2022 can be explained by a new user using a large number of animals.



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

In 2023, the uses reported in the different actual severities are 6% in Non-recovery, 31% in Mild, 52% in Moderate and 11% in Severe. Looking at the distribution of the severities over the years 2015 and forward, there are minor fluctuations in all categories: Non-recovery: 2 to 6 %, with a mean of 4; Mild: 24 to 37 % with a mean of 30 %; Moderate, 52 to 63 % with a mean of 57 %, and in Severe: 8 to 12 % with a mean of 10 %. Compared to the last year in the series, Moderate in 2023 is lower than in 2022, whereas all other severities have increased. In order to establish any trends, more data from coming years are needed.



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

General information

The Swedish National Committee for the Protection of Animals Used for Scientific Purposes drives the Swedish national work with the 3Rs, with the Swedish 3Rs Center as its executive body. The main task for the 3Rs Center is to carry out the projects decided by the National Committee and to support 3Rs work nationally. Below follow some activities that National Committee and Swedish 3Rs Center has had during the year. For more information please see the National Committee Annual Report for 2023.

Meetings

Seminar with focus on the human brain
At the beginning of the year, the 3Rs Center held a seminar on non-animal methods for studying the human brain. The Joint Research Center of the EU Commission presented their systematic work to collect and compile available non-animal methods used within different disease areas. Researchers from Swedish universities presented their projects and methods with focus on the 3Rs, and a representative for the Swedish Society for Neuroscience presented their activities. The day ended with a panel discussion about possibilities and obstacles for implementation of non-animal methods within research on the human brain and its diseases.

Webinars
During the year, the 3Rs Center arranged four webinars on research based on non-animal methods. The topics were the Swedish Research Council's 3Rs project grant, organ-on-chip methods, cell culture using animal-free components, and 3D-printing of tissues.
The 3Rs Center also organized five webinars focusing on animal welfare: for example, *Systematic literature reviews to use existing research to reduce the number of animals needed in experiments*; and *Using alternative methods for health assessment of laboratory animals*.

The annual meeting for animal welfare bodies
A recurring event is the annual meeting for the animal welfare bodies. This year's theme was change management, communication and refined handling of mice; with a presentation on why one should avoid lifting mice by the tail, a presentation of the National Committee's statement

"Don't lift mice in the tail!", a workshop on the implementation of refined mouse handling, and a presentation on how AstraZeneca works with this matter globally.

National meeting for animal technicians
The 3Rs Center held an on-site meeting for animal technicians during the International Laboratory Animal Technician Week.

Other meetings
The 3Rs Center and the National Committee also participated in other meetings such as the World Congress on Alternatives and Animal Use in the Life Sciences in Canada, the Scand-LAS Symposium in Sweden, the Danish 3Rs Symposium, and a Nordic meeting on zebrafish research.

Networking and collaboration

National networks
The 3Rs Center's network with Swedish animal technicians and animal caretakers has expanded to nearly 100 members. It is an important channel to share insights and learnings. Further, the continuous work with the national replace network has proceeded, and the network now has more than 400 members.

European National Committee Network
For a few years, the Swedish National Committee has participated in a network for all committees in Europe - the European NC Network. In 2023, Poland hosted the annual meeting, where the participants discussed current topics and questions that they have in common.

The European network for 3Rs organisations: EU3Rnet
The 3Rs Center and the National Committee are included in the EU3Rnet's steering committee and in two of the working groups dealing with training and implementation. During the year, the 3Rs Center has participated in two meetings with the steering committee and further meetings in the various working groups that are now starting up their activities. The 3Rs Center is also involved in the network's COST (European Cooperation in Science and Technology) project IMPROVE.

3Rs across Europe
The 3Rs Centre in the United Kingdom, the NC3Rs, has initiated a smaller and more informal network. Participants from 3Rs organisations in Sweden, Denmark, Germany, Switzerland and the Netherlands meet to share knowledge, and collaborate when possible. In June, the network jointly organised a webinar series on Culture of care with a focus on implementation and success factors.

National Contact Point for the PARERE network
Since 2021, the Swedish 3Rs Center has the role of contact point for the PARERE-network – EURL ECVAM Network for Preliminary Assessment of Regulatory Relevance. During 2023, the Center participated in two meetings; the annual meeting at the EU Commission's Joint Research Center and an online meeting in June, where a new algorithm that can be used for chemical safety testing and assessment based on non-animal methods was discussed.

European Research Area
During the fall, the 3Rs Center participated in a work initially submitted by representatives from the European Commission and the Netherlands to put focus on replacing animal experimentation in the upcoming EU political agenda for 2025–2027 within the European Research Area (ERA).

Activities for students and teachers

Educational Material
The 3Rs Center has produced a wide range of educational material specifically aimed at the upper secondary school. A large part of the work was launched in 2022 and the use and marketing of the material through for example web-based resources for teachers and social media has continued during 2023.

Educational Events
In Sweden, there are a number of secondary school courses for future animal technicians and the 3Rs Center collaborates with teachers at the upper secondary level to educate their students on the 3Rs. In 2023, the 3Rs Center offered two half-day education events, focused on refinement and modern handling techniques as well as methods that can replace animal experimentation today and tomorrow.

Researcher's Night
The 3Rs Center participated 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. The aim is to increase the general knowledge on the subject.

Writing Competition
In February, the 3Rs Center organised a national writing competition for the upper secondary school, encouraging students to write a story or a poem about a world without animal experimentation, and reflect upon how such a reality would differ from todays. The competition aimed to spark interest in the 3Rs in general, and non-animal methods in particular, among young people. An additional aim was to raise awareness of the Center's educational material on research, animal experimentation and the 3Rs. The winning student's class was given an award of 15 000 SEK to the class savings.

Collaborations
During 2023, the 3Rs Center started a collaboration with the organization Young Researchers (Unga forskare) that works to increase interest in science and technology among students.
The Center also initiated collaboration with Upptech Jönköping Science Center in order to meet students and teachers in primary and secondary schools. In October, a meeting was held with teachers from the secondary school in Jönköping municipality exchanging knowledge and ideas on the 3Rs.

Materials and resources

The 3Rs Center has produced different materials and resources during the year in the form of publications, recommendations and films.

Publications
During 2023, the 3Rs Center has published four editions of the digital letter Focus on the 3Rs: *Methods to replace animal experimentation, The role of the animal technician, Methods to reduce the number of animals used in research,* and *The 3Rs Center's impact on the development of Replacement in Sweden*. The 3Rs Center also published articles with good examples of research and development within the 3Rs with examples from Swedish universities, companies and authorities; as well as an article on the projects that received the 3Rs grant from the Swedish Research Council in 2022.

Recommendations
During the year, the 3Rs Center has worked on several recommendations: *Refined mouse handling, Support in choosing euthanasia method for mice, Group and solitary housing of rabbits, Interior for laboratory fish* and *Acclimatization of laboratory animals.*

Good examples of Refinement
The 3Rs Center has continued its work to collect and disseminate good examples of refinement from Swedish users. The aim is to promote and inspire further work on refining animal experimentation. The compilation is directed to anyone who works with laboratory animals, and the report was published at the end of 2023.

Films to promote non-animal methods
The 3Rs Center published a film on how researchers have replaced animal experimentation in drug development by using cell models to inspire other researchers to make transitions to other research models. Another film explaining how pluripotent stem cells can replace rodents in research on neurological diseases was created in 2023 and launched in early 2024.

Strategic work

The National Committee provided input to the government's proposition on research and innovation focusing on the 3Rs principle as a means to increase research quality. They also specified the need for increased funding for research on all 3Rs, for validation and for collaboration between stakeholders. All this is crucial to replace, reduce and refine animal experimentation.

Strategy to replace animal experiments
The National Committee continued the work with compiling a strategy to replace animal experiments. The aim of the strategy is primarily to direct the work of the National Committee in replacement related issues, but it will also be used to inspire other stakeholders. The work with the strategy is ongoing.

Funding
In the Swedish government's budget proposition for 2024, the dedicated funding to the 3Rs Center was removed. Due to this, the Swedish Board of Agriculture notified nine of the twelve positions at the 3Rs Center. This also means that the conditions for the work of the National Committee will change. In order to adjust to a smaller budget, the National Committee has identified the most important target groups for the 3Rs Center's work in 2024 and has decided on a significantly reduced operational plan.

Impacts on statistics

It is difficult to measure whether the actions that the 3Rs Center perform have reduced the number of animals used in Swedish research. However, the National Committee and the 3Rs Center are continuously striving towards informing and inspiring researchers to find new ways to replace, reduce and refine animal use.

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

Purposes

The only use of the 'other' categories that has been reported in a significant proportion under the 'Purposes' category in 2023 was within *Other efficacy and tolerance testing: Efficacy studies of drug candidates for neurological diseases/conditions and infectious conditions*, with 830 uses (79%) in total; 414 mice, 352 rats and 64 rabbits.

Species

Other carnivores

In 2023, 102 uses of carnivores were recorded as Other carnivores (16%). This is similar to 2022, with 109 uses (17%). Used species in 2023 were brown bear (*Ursus arctos*, 76), wolverine (*Gulo gulo*, 8), lynx (*Lynx lynx*, 3), wolf (*Canis lupus*, 5) and weasel (*Mustela nivalis,* 10). All uses of bears and weasels, as well as 6 wolverines were used in *Preservation of Species*. The wolves and the lynx as well as 2 wolverines were used in *Basic Research* with subcategory *Ethology/Animal Behaviour/Animal Biology*.

Used species in 2022 were brown bear (76), raccoon dog (*Nyctereutes procyonoides*, 18), wolverine (5), lynx (7), and wolf (3). All uses of bears, raccoon dogs, lynxes and wolverines were in *Preservation of Species*. The wolves were used in *Basic Research* with subcategory *Ethology/Animal Behaviour/Animal Biology*.

In 2021, uses were reported for brown bear (74), American mink (*Neovison vison,* 72), raccoon dog (29), European polecat (*Mustela putorius*, 26), wolverine (11), lynx (2), and wolf (1). All uses of bears, wolverine, lynx and wolf were in *Preservation of Species*. The raccoon dogs were used in *Protection of the natural environment in the interests of the health or welfare of human beings or animals*, the polecats in *Basic Research, Nervous System* and the minks in *Translational and applied research, Human Infectious Disorders*.

Other birds

In 2023, 7 193 uses (87 %) of the birds were reported as Other birds. The most common species was the collared flycatcher (*Ficedula albicollis,* 2 349), followed by Eurasian blue tit (*Cyanistes caeruleus,* 948), great tit (*Parus major,* 813), European pied flycatcher (*Ficedula hypoleuca,* 793), common blackbird (*Turdus merula,* 359), mallard (*Anas platyrhynchos,* 276), great reed warbler (*Acrocephalus arundinaceus,* 233), Japanese quail (*Coturnix japonica,* 124), common eider (*Somateria mollissima,* 169), European pied flycatcher x collared flycatcher (hybrid) (82), velvet scoter (*Melanitta fusca,* 68) and Eurasian teal (*Anas crecca,* 63). Almost all uses were within *Basic research: Ethology/Animal Behaviour/Animal Biology.* However, uses were also reported in *Basic Research, Endocrine System/Metabolism (168), Protection of the natural environment in the interests of the health or welfare of human beings or animals* (155) and *Preservation of species* (3).

5 179 uses (86 %) of the birds were reported as Other birds in 2022. The most common species was the collared flycatcher (2,431), followed by Eurasian blue tit (1 334), European pied flycatcher (572), great tit (377), common blackbird (283), Siberian jay (*Perisoreus infaustus,* 174), European pied flycatcher x collared flycatcher (hybrid) (6) and Montagu's harrier (*Circus pygargus,* 2). All uses were within *Basic research: Ethology/Animal Behaviour/Animal Biology.*

In 2021, 82% of the uses (5 526) of the birds were reported as *Other birds*. The largest uses were then found in great tit (1,300), European robin (*Erithacus rubecula,* 433), zebra finch (*Taeniopygia guttata,* 372), European pied flycatcher (397), willow warbler (*Phylloscopus trochilus,* 363), Eurasian blue tit (292), great reed warbler (221), common blackbird (158), Japanese quail (92) and song thrush (*Turdus philomelos,* 78). Main uses were within *Basic research: Ethology/Animal Behaviour/Animal Biology* (5123), some in *Endocrine System/Metabolism* (167), *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (221) and 7 uses in *Preservation of species*.

Other fish

In 2023, 18 604 fish (29 %) were reported as *Other fish*. Most uses were reported of Atlantic mackerel (*Scomber scombrus,* 4 000), Atlantic herring (*Clupea harengus*, 2 845), European perch (*Perca fluviatilis*, 2 632), three-spined stickleback (*Gasterosteus aculeatus*, 2 052), common bleak (*Alburnus alburnus*, 1 963), roach (*Rutilus rutilus*, 1 955), minnow (*Phoxinus phoxinus*, 664), white bream (*Blicca bjoerkna)*/common bream (*Abramis brama*)/white bream x common bream (hybrid) (590), pike (*Esox lucius*, 250), Nile tilapia (*Oreochromis niloticus,* 201), alpine bullhead (*Cottus poecilopus,* 168) and European bullhead (*Cottus gobio,* 162). Fish in this category were used in *Protection of the natural environment in the interests of the health or welfare of human beings or animals* (7 339), *Preservation of species* (5 154), *Translational and applied research, Non-regulatory toxicology and ecotoxicology* (4 415), *Higher education* (1188), *Translational and applied research, Animal Welfare* (288), *Basic research, Cardiovascular Blood and Lymphatic System* (60) and *Basic research, Ethology / Animal Behaviour /Animal Biology* (160).

In 2022, 32 273 (45%) of the reported uses of fish were *Other fish*, a decrease proportionally speaking compared to 2021 with 42 803 uses (57%). In 2022, most uses were reported of roach (6 230), Atlantic mackerel (5 000), European perch (4 538), Atlantic herring (2 865), common bleak (2 805), gobies (*Pomatoschistus,* 2 072), minnow (1 481), Pygmy halfbeak (*Dermogenys collettei,* 1 204), pike (1 105), white bream x common bream (hybrid, 590), Atlantic cod (*Gadus morhua*, 546), three-spined stickleback (466).

In 2021, most Other fish are reported as roach (10 396), European perch (6 026), three-spined stickleback (5 770), common bleak (5 136), Atlantic herring (3 664), minnow (2 386) and white bream (2 338).

Other amphibians

99 % (1 097) of the amphibian use in 2023 was reported as Other amphibians. Species used were the Iberian ribbed newt (*Pleurodeles waltl,* 885), followed by pool frog (*Pelophylax lessonae,* 109), edible frog (*Pelophylax kl. esculentus,* 44), common toad (*Bufo bufo,* 42), eastern newt (*Notophthalmus viridescens,* 15) and moor frog (*Rana arvalis,* 2). They were used in *Basic research: Nervous System* (13 eastern newts and 698 Iberian ribbed newts), *Basic research: Cardiovascular Blood and Lymphatic System* (2 eastern newts and 187 Iberian ribbed newts), *Preservation of species* (109 pool frogs) and *Ethology/Animal Behaviour/Animal Biology* (44 edible frogs, 42 common toads and 2 moor frogs).

In 2022, 99 % (3,004) of the amphibian use were reported as Other amphibians, all used in *Basic research: Ethology/Animal Behaviour/Animal Biology* (1,454), *Nervous System* (961), *Cardiovascular Blood and Lymphatic System* (501) and *Immune System* (88). The most frequently used species in 2022 was the Iberian ribbed newt (1 550), followed by moor frog (946), common toad (324), and edible frog (184).

In 2021, most uses were reported of Iberian ribbed newt (1 846) and the remaining 25 were eastern newt. All uses were reported in *Basic research*; more specifically in *Nervous System* (1 264) and *Cardiovascular Blood and Lymphatic System* (607).

(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 during 2023.

(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 have been no cases where the severe classification was exceeded, neither pre-authorised nor unexpected, in SE up to this date.

Norway

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

The numbers, purposes and severities of animals used in 2023 are mainly within the trends known from previous years. Notable changes are described below.

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

- 1 364 pigs were used in research projects in 2023, which is an increase from 2022, when 737 pigs were used. 810 of the pigs were used in one single research project where an autologous vaccine was tested in two commercial pig herds.

- 211 privately owned dogs were used in research projects in 2023, most of them with a mild severity. The research areas include breeding/genetic screening, dog feed composition, autologous dog vaccines and dog dental health issues.

- 2 168 domestic fowl were used in 2023, an increase from 1 456 in 2022. Two feeding trials in particular contribute to this increase.

- The use of both sheep and cattle was reduced in 2023 compared to 2022, while no goats were used in 2023. There are relatively few projects using these species, and single projects have a significant impact on the statistics.

- The use of rats was reduced in 2023 compared to 2022, from 3 397 in 2022 to 2 740 in 2023. The decrease in rat use follows a downward trend over time and could be attributed to space and economy limitations combined with the availability of more specialised mouse models. Mouse use has increased from 53 817 in 2022 to 55 102 in 2023. The increased mouse use could represent a replacement of rats, but this is uncertain.

- The number of salmonids (salmon, trout, chars and graylings) increased from 2022 to 2023, but lower than in 2021. The increase from 2022 til 2023 was about 140 000. As previous years a few projects with a high number of fish have a large impact on the statistics. Projects with the range of 10 000-50 000 animals had the largest impact on the numbers, i.e. 8 such projects in 2023 and 4 I 2022. , resulting in about 98 000 more salmonids in 2023. Also, there were 3 projects with the range of 180-600 000 animals in 2023 and 2 such projects in 2022, resulting in about 60 000 more salmonids in 2023. There were no projects using between 50 and 180 000 salmonides.

- The number of Other fish increased from 2022 to 2023. The main contribution to this increase is one experiment with self feeding halibut larvae in 2023.

- The number of Zebrafish increased from 2022 to 2023. Although the number of projects were equal (n=20), 2023 had a higher number of projectss using more than 1 000 fish (n=5),

compared to 2022 (n=1). Among these projects in 2023 (where more than 1 000 zebrafish were used), especially two contributed to this increase, one modeling severe neurological disorders in vivo for high-throughput drug discovery, and one studying evolution and mechanisms of thermal performance.

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

The proportion of mild (63%) was higher than moderate (34%) in 2023. This is an increase of mild severity, and a reduction of moderate severity compared to 2021 and 2022, where the proportion of moderate were higher than mild. The increase in mild actual severity is a result of fewer salmon used in moderately severe experiments for the removal of salmon lice in 2023, compared to 2021 and 2022.

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

Norway have continued the efforts from previous years. See 2022 narrative.

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

We find that the other categories does not contribute to a significant proportion of 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:

We are not aware of any such use of animals reported. We have not registered any applications for such use and in decisions (approval of applications) we include the following text: "This approval is given on the condition that there are no relevant methods that are less burdensome for animals (refined methods), use fewer animals or methods without the use of animals (alternative methods). If possibilities for refinement, reduction or the use of alternatives to animals should become available during the approval period, the person responsible for the experiment (applicant) is responsible for ensuring that such methods are used". For the replacement of fish in batch testing of fish vaccines, apart for the already replaced IPNV-test, the validation work is ongoing for several vaccines.

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

- **Species:** none
- **Numbers of animals:** none
- **Whether exceeding the 'severe' classification was pre-authorised or not: -**
- **Details of the use: -**
- **Reasons why the 'severe' classification was exceeded:**

IV. Member States comparative tables for 2023

Introduction

Four Member State comparative tables are provided for 2023 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 1.1: 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	Czechia	Denmark	Estonia	Finland	France	Germany	Greece	Hungary	Ireland	Italy
Mammals															
Rodents															
Mice	148,830	231,411	1,156	23,412	2,450	62,439	137,522	2,146	43,037	870,958	845,864	13,342	43,391	86,871	212,438
Rats	2,447	10,776	1,642	3,819	0	20,044	23,352	245	7,214	128,162	98,568	2,335	26,148	8,440	74,003
Guinea-Pigs	108	9,147	40	25	0	1,954	1,639	0	0	30,596	7,805	3	2,966	1,188	14,385
Hamsters (Syrian)	137	2,010	0	0	0	459	106	0	309	7,025	1,854	0	368	24	472
Hamsters (Chinese)	0	35	0	0	0	0	0	0	0	55	0	0	0	0	12
Mongolian gerbil	61	17	0	0	0	0	0	0	0	489	2,076	0	0	0	50
Other rodents	165	146	0	0	0	1,915	0	0	894	814	1,815	0	0	11	351
Rabbits															
Rabbits	938	61,795	10	40	0	3,048	1,869	42	149	176,673	64,508	58	1,004	1,852	5,957
Carnivores															
Cats	15	214	0	0	2	129	0	0	232	367	269	0	3	8	0
Dogs	87	268	100	0	7	197	267	0	698	2,593	1,100	0	211	54	450
Ferrets	0	0	0	0	0	3	0	0	0	102	78	0	0	175	35
Other carnivores	0	11	0	0	0	4	0	0	49	4,878	56	0	0	0	0
Farm animals															
Horses, donkeys and cross-breeds	60	179	0	0	0	95	32	0	47	54	397	0	18	5	0
Pigs	1,915	4,291	25	44	0	1,413	12,846	4	320	10,630	9,363	192	1,311	752	1,987
Goats	30	8	0	0	0	24	2	0	0	541	105	0	0	26	7
Sheep	149	330	0	3	0	167	74	112	263	3,129	2,001	0	132	3,088	68
Cattle	999	498	0	0	0	1,520	4,324	493	677	1,896	2,284	0	0	1,892	421
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	36	2	0	0	0	0
Marmoset and tamarins	0	0	0	0	0	0	0	0	0	225	98	0	0	0	0
Cynomolgus monkey	0	0	0	0	0	0	0	0	0	2,065	1,320	0	6	0	142
Rhesus monkey	0	6	0	0	0	0	0	0	0	14	55	0	0	0	1
Baboons	0	0	0	0	0	0	0	0	0	31	3	0	0	0	0
Squirrel monkey	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Other mammals															
Other mammals	78	272	0	0	0	29	21	0	0	691	1,895	0	0	0	6
Birds															
Domestic fowl	879	29,153	240	1,049	0	25,029	2,659	116	2,601	65,913	8,965	500	25,365	37	31,975
Turkey	75	1,206	0	0	0	364	0	0	1	14,317	2,199	0	755	3	402
Other birds	1,257	3,941	0	0	0	3,495	420	28	942	10,900	8,316	0	2,359	6	157
Reptiles															
Reptiles	6	8	0	0	0	360	107	0	0	1,969	137	0	376	0	0
Amphibians															
Rana	0	806	200	0	0	0	0	0	240	6	1,332	0	0	0	0
Xenopus	657	98	0	0	0	204	90	0	0	6,132	1,058	0	0	4	174
Other amphibians	5,899	1,071	276	0	0	2	102	0	242	176	2,309	0	0	0	0
Fish															
Zebra fish	22,082	59,591	0	24	0	4,842	5,396	0	6,035	49,883	82,307	350	769	0	13,606
Sea bass	0	27	0	0	0	0	5	0	0	15,162	0	3,400	0	0	747
Salmon, trout, chars and graylings	1,359	593	0	0	0	7,292	12,733	0	3,345	27,308	10,013	0	0	2,068	1,189
Guppy, swordtail, molly, platy	0	0	0	0	0	78,568	0	0	0	0	328	0	395	0	735
Other fish	12,363	3,980	0	530	0	8,322	533	120	1,166	56,551	23,032	4,035	1,282	135	540
Cephalopods															
Cephalopods	0	10	0	0	0	0	0	0	0	820	70	0	0	0	9
Totals															
Total	**200,596**	**421,898**	**3,689**	**28,946**	**2,459**	**221,918**	**204,099**	**3,306**	**68,461**	**1,491,162**	**1,181,582**	**24,215**	**106,859**	**106,639**	**360,319**
%	**2.5**	**5.3**	**0**	**0.4**	**0**	**2.8**	**2.6**	**0**	**0.9**	**18.7**	**14.8**	**0.3**	**1.3**	**1.3**	**4.5**

Table 1.2: 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	1,281	3,272	3,073	0	110,796	48,282	66,724	44,458	7,407	3,772	3,351	399,035	143,286	**3,560,004**	**44.6**
Rats	256	1,055	0	0	80,763	2,740	12,568	3,261	3,448	4,253	96	45,985	15,206	**576,826**	**7.2**
Guinea-Pigs	0	15	0	0	4,681	252	5,781	0	2,748	134	0	8,169	296	**91,932**	**1.2**
Hamsters (Syrian)	0	0	0	0	1,655	30	12	0	50	0	0	999	13	**15,523**	**0.2**
Hamsters (Chinese)	0	0	0	0	0	0	0	0	0	0	0	9	0	**111**	**0**
Mongolian gerbil	0	0	0	0	72	0	0	0	0	14	0	33	0	**2,812**	**0**
Other rodents	0	0	0	0	1,088	30	3,087	104	0	0	0	695	1,750	**12,865**	**0.2**
Rabbits															
Rabbits	0	120	0	0	17,277	12	718	2	146	83	0	10,192	2,562	**349,055**	**4.4**
Carnivores															
Cats	334	0	0	0	4	0	0	0	0	21	0	94	148	**1,840**	**0**
Dogs	0	0	0	0	546	215	58	0	0	0	0	1,112	389	**8,352**	**0.1**
Ferrets	0	0	0	0	331	0	0	0	0	0	0	44	0	**768**	**0**
Other carnivores	0	0	0	0	19	79	0	0	0	0	0	6	52	**5,154**	**0.1**
Farm animals															
Horses, donkeys and cross-breeds	0	0	0	0	314	196	328	0	0	0	0	223	382	**2,330**	**0**
Pigs	8	57	0	0	8,639	1,364	1,056	110	22	0	7	12,318	2,739	**71,413**	**0.9**
Goats	0	0	0	0	40	0	6	0	0	0	0	170	16	**975**	**0**
Sheep	41	5	0	0	1,721	164	191	20	1	35	0	1,334	176	**13,204**	**0.2**
Cattle	2	0	0	0	3,372	238	571	0	0	0	0	824	454	**20,465**	**0.3**
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	0	0	0	0	**38**	**0**
Marmoset and tamarins	0	0	0	0	5	0	0	0	0	0	0	0	0	**328**	**0**
Cynomolgus monkey	0	0	0	0	0	0	0	0	0	0	0	273	20	**3,826**	**0**
Rhesus monkey	0	0	0	0	89	0	0	0	0	0	0	0	0	**165**	**0**
Baboons	0	0	0	0	0	0	0	0	0	0	0	0	0	**34**	**0**
Squirrel monkey	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**	**0**
Other mammals															
Other mammals	0	0	0	0	902	399	165	26	0	0	0	776	494	**5,754**	**0.1**
Birds															
Domestic fowl	0	0	0	0	22,000	2,214	2,959	0	3,354	341	536	104,360	1,110	**331,355**	**4.2**
Turkey	0	0	0	0	110	0	432	0	0	0	0	0	0	**19,864**	**0.2**
Other birds	0	61	0	0	18,955	7,983	3,533	0	20	37	0	5,934	7,066	**75,410**	**0.9**
Reptiles															
Reptiles	0	0	0	0	0	0	70	0	0	0	0	306	0	**3,339**	**0**
Amphibians															
Rana	0	0	0	0	0	0	0	0	0	0	0	0	0	**2,584**	**0**
Xenopus	0	0	0	0	706	10	0	14	0	0	0	161	2	**9,310**	**0.1**
Other amphibians	0	0	0	0	0	0	1,155	0	0	0	0	3,754	1,045	**16,031**	**0.2**
Fish															
Zebra fish	0	351	123	0	2,757	24,599	5,597	14,683	1,275	0	0	31,758	23,166	**349,194**	**4.4**
Sea bass	0	0	0	25,567	1,029	0	0	6,085	0	0	0	337,416	0	**389,438**	**4.9**
Salmon, trout, chars and graylings	0	0	0	0	1,000	1,305,187	68,046	4,299	0	0	240	14,007	11,628	**1,470,307**	**18.4**
Guppy, swordtail, molly, platy	0	0	0	0	267	0	0	0	0	0	0	42	0	**80,335**	**1**
Other fish	0	0	0	13,104	68,782	160,315	3,625	4,181	0	0	0	95,029	18,604	**476,229**	**6**
Cephalopods															
Cephalopods	0	0	0	0	77	1	0	12	0	0	0	6,056	0	**7,055**	**0.1**
Totals															
Total	**1,922**	**4,936**	**3,196**	**38,671**	**347,997**	**1,554,310**	**176,682**	**77,255**	**18,471**	**8,690**	**4,230**	**1,081,114**	**230,604**	**7,974,226**	**100**
%	**0**	**0.1**	**0**	**0.5**	**4.4**	**19.5**	**2.2**	**1**	**0.2**	**0.1**	**0.1**	**13.6**	**2.9**	**100**	

Table 2.1: 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	Czechia	Denmark	Estonia	Finland	France	Germany	Greece	Hungary	Ireland	Italy
Mammals															
Rodents															
Mice	149,331	233,055	1,536	23,412	2,450	62,948	138,861	2,146	43,123	876,534	863,879	13,404	44,556	86,871	212,973
Rats	2,495	11,656	2,472	3,819	0	20,061	23,574	245	7,256	129,669	100,543	2,335	26,488	8,440	74,307
Guinea-Pigs	108	9,179	40	45	0	1,979	1,639	0	0	30,670	7,863	3	3,028	1,188	14,444
Hamsters (Syrian)	137	2,011	0	0	0	459	106	0	309	7,056	1,854	0	368	24	472
Hamsters (Chinese)	0	35	0	0	0	0	0	0	0	55	0	0	0	0	12
Mongolian gerbil	61	17	0	0	0	0	0	0	0	489	2,090	0	0	0	50
Other rodents	165	146	0	0	0	2,005	0	0	894	814	1,898	0	0	11	351
Rabbits															
Rabbits	994	61,799	300	40	0	3,391	1,870	42	149	178,030	67,524	58	1,043	1,852	6,915
Carnivores															
Cats	15	214	0	0	2	216	0	0	232	951	544	0	3	24	0
Dogs	91	705	100	0	7	459	473	0	767	4,080	2,550	3	255	66	518
Ferrets	0	0	0	0	0	3	0	0	0	108	78	0	0	175	40
Other carnivores	0	11	0	0	0	4	0	0	49	4,878	56	0	0	0	0
Farm animals															
Horses, donkeys and cross-breeds	76	232	0	6	0	117	33	0	93	328	491	0	95	10	0
Pigs	2,043	4,463	25	44	0	1,504	12,997	4	321	10,907	9,604	196	1,400	752	2,015
Goats	30	9	0	0	0	32	2	0	0	736	127	0	0	26	7
Sheep	149	359	120	27	0	487	74	112	473	3,836	2,101	0	142	3,280	122
Cattle	1,069	584	0	0	0	1,578	4,608	493	738	2,850	2,465	0	0	2,855	421
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	36	5	0	0	0	0
Marmoset and tamarins	0	0	0	0	0	0	0	0	0	275	106	0	0	0	0
Cynomolgus monkey	0	0	0	0	0	0	0	0	0	3,077	1,478	0	12	0	165
Rhesus monkey	0	39	0	0	0	0	0	0	0	24	73	2	0	0	1
Baboons	0	0	0	0	0	0	0	0	0	39	3	0	0	0	0
Squirrel monkey	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
Other mammals															
Other mammals	78	338	0	0	0	29	21	0	136	726	2,010	0	0	0	257
Birds															
Domestic fowl	879	29,304	790	1,139	0	25,085	2,659	116	2,608	65,944	9,262	739	25,422	37	32,038
Turkey	75	1,206	0	0	0	366	0	0	1	14,317	2,221	0	755	3	402
Other birds	1,257	4,176	0	0	0	3,538	420	28	942	10,979	8,437	0	2,359	6	188
Reptiles															
Reptiles	6	235	0	0	0	360	124	0	0	2,344	140	0	376	0	0
Amphibians															
Rana	0	806	200	0	0	0	0	0	240	6	1,332	0	0	0	0
Xenopus	823	181	0	0	70	204	490	0	0	6,969	1,687	0	0	50	205
Other amphibians	5,899	1,071	276	0	0	2	109	0	242	191	2,309	0	0	0	0
Fish															
Zebra fish	22,082	59,591	0	24	0	4,842	6,427	0	6,035	49,883	82,443	500	2,416	0	13,606
Sea bass	0	27	0	0	0	0	5	0	0	15,162	0	3,438	0	0	747
Salmon, trout, chars and graylings	1,359	715	0	0	0	7,332	12,817	0	3,345	27,308	14,550	0	0	2,068	1,234
Guppy, swordtail, molly, platy	0	0	0	0	0	78,703	0	0	0	0	328	0	395	0	735
Other fish	12,363	4,411	0	530	0	8,359	533	120	1,166	57,304	23,051	4,523	1,686	135	540
Cephalopods															
Cephalopods	0	10	0	0	0	0	0	0	0	820	71	0	0	0	9
Totals															
Total	**201,585**	**426,585**	**5,859**	**29,086**	**2,529**	**224,063**	**207,842**	**3,306**	**69,119**	**1,507,403**	**1,213,173**	**25,201**	**110,799**	**107,873**	**362,774**
%	**2.5**	**5.3**	**0.1**	**0.4**	**0**	**2.8**	**2.6**	**0**	**0.9**	**18.7**	**15**	**0.3**	**1.4**	**1.3**	**4.5**

Table 2.2: All uses (first use and all subsequent reuses) of animals for research, testing, routine production and educational purposes by species and Member State (Part2)

	Latvia	Lithuania	Luxembourg	Malta	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Slovenia	Spain	Sweden	Total	%
Mammals															
Rodents															
Mice	1,281	3,272	3,073	0	111,626	48,367	67,454	44,458	7,657	3,772	3,783	399,873	143,866	**3,593,561**	**44.5**
Rats	256	1,055	0	0	81,415	2,740	12,568	3,309	3,498	4,307	102	46,272	15,245	**584,127**	**7.2**
Guinea-Pigs	0	15	0	0	4,711	252	5,796	0	2,748	134	0	8,436	296	**92,574**	**1.1**
Hamsters (Syrian)	0	0	0	0	1,655	30	12	0	50	0	0	999	13	**15,555**	**0.2**
Hamsters (Chinese)	0	0	0	0	0	0	0	0	0	0	0	9	0	**111**	**0**
Mongolian gerbil	0	0	0	0	72	0	0	0	0	14	0	33	0	**2,826**	**0**
Other rodents	0	0	0	0	1,088	30	3,129	104	0	0	0	695	1,805	**13,135**	**0.2**
Rabbits															
Rabbits	0	120	0	0	17,310	12	1,067	2	146	83	64	15,644	2,562	**361,017**	**4.5**
Carnivores															
Cats	334	0	0	0	138	0	0	0	0	21	0	1,035	148	**3,877**	**0**
Dogs	0	0	0	0	947	215	58	14	0	0	0	1,491	401	**13,200**	**0.2**
Ferrets	0	0	0	0	337	0	0	0	0	0	0	44	0	**785**	**0**
Other carnivores	0	0	0	0	19	79	0	0	0	0	0	6	102	**5,204**	**0.1**
Farm animals															
Horses, donkeys and cross-breeds	0	0	0	0	354	200	346	4	3	0	0	322	444	**3,154**	**0**
Pigs	8	57	0	0	8,882	1,364	1,056	110	22	0	7	12,390	2,767	**72,938**	**0.9**
Goats	0	0	0	0	206	0	6	40	0	0	0	360	24	**1,605**	**0**
Sheep	41	5	0	0	1,736	164	195	30	246	35	3	1,608	176	**15,521**	**0.2**
Cattle	2	0	0	0	4,215	362	576	12	3	0	0	1,362	502	**24,695**	**0.3**
Non-human primates															
Prosimians	0	0	0	0	0	0	0	0	0	0	0	0	0	**41**	**0**
Marmoset and tamarins	0	0	0	0	5	0	0	0	0	0	0	0	0	**386**	**0**
Cynomolgus monkey	0	0	0	0	22	0	0	0	0	0	0	295	39	**5,088**	**0.1**
Rhesus monkey	0	0	0	0	117	0	0	0	0	0	0	0	15	**271**	**0**
Baboons	0	0	0	0	0	0	0	0	0	0	0	0	0	**42**	**0**
Squirrel monkey	0	0	0	0	0	0	0	0	0	0	0	0	0	**8**	**0**
Other mammals															
Other mammals	0	0	0	0	902	801	165	26	0	0	0	778	560	**6,827**	**0.1**
Birds															
Domestic fowl	0	0	0	0	22,010	2,214	2,959	0	3,374	341	536	104,446	1,110	**333,012**	**4.1**
Turkey	0	0	0	0	175	0	432	0	3	0	0	0	0	**19,956**	**0.2**
Other birds	0	61	0	0	19,126	7,988	3,533	0	20	37	0	5,934	7,193	**76,222**	**0.9**
Reptiles															
Reptiles	0	0	0	0	0	0	72	0	20	0	0	306	191	**4,174**	**0.1**
Amphibians															
Rana	0	0	0	0	0	0	0	0	0	0	0	0	0	**2,584**	**0**
Xenopus	0	0	0	0	867	10	0	14	0	0	0	185	6	**11,761**	**0.1**
Other amphibians	0	0	0	0	0	0	1,155	0	0	0	0	3,754	1,097	**16,105**	**0.2**
Fish															
Zebra fish	0	351	123	0	2,757	24,999	5,597	14,869	1,275	0	0	31,758	23,166	**352,744**	**4.4**
Sea bass	0	0	0	25,567	1,029	0	0	7,277	0	0	0	337,536	0	**390,788**	**4.8**
Salmon, trout, chars and graylings	0	0	0	0	1,000	1,308,187	68,046	4,299	0	0	240	14,007	11,628	**1,478,135**	**18.3**
Guppy, swordtail, molly, platy	0	0	0	0	267	0	0	0	0	0	0	42	0	**80,470**	**1**
Other fish	0	0	0	13,104	68,807	160,315	4,012	4,271	0	0	0	103,194	18,604	**487,028**	**6**
Cephalopods															
Cephalopods	0	0	0	0	77	1	0	24	0	0	0	6,056	0	**7,068**	**0.1**
Totals															
Total	**1,922**	**4,936**	**3,196**	**38,671**	**351,872**	**1,558,330**	**178,234**	**78,863**	**19,065**	**8,744**	**4,735**	**1,098,870**	**231,960**	**8,076,595**	**100**
%	**0**	**0.1**	**0**	**0.5**	**4.4**	**19.3**	**2.2**	**1**	**0.2**	**0.1**	**0.1**	**13.6**	**2.9**	**100**	

Table 3.1: 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	Bulgaria	Croatia	Cyprus	Czechia	Denmark	Estonia	Finland	France	Germany	Greece	Hungary	Ireland	Italy	Latvia	Luxembourg	Netherlands	Norway	Poland	Portugal	Romania	Slovenia	Spain	Sweden	Total	%
Mice	No	7,676	18,901	0	0	0	4,892	2,750	0	3,533	40,627	122,020	7,694	582	203	2,800	46	0	7,520	2,765	704	1,238	0	0	16,836	5,483	**246,270**	**99.9**
	Yes	0	1	0	0	0	0	0	0	0	0	102	20	0	0	146	0	0	0	0	0	0	0	0	0	15	**284**	**0.1**
	Total	**7,676**	**18,902**	**0**	**0**	**0**	**4,892**	**2,750**	**0**	**3,533**	**40,627**	**122,122**	**7,714**	**582**	**203**	**2,946**	**46**	**0**	**7,520**	**2,765**	**704**	**1,238**	**0**	**0**	**16,836**	**5,498**	**246,554**	**100.0**
Rats	No	110	281	0	0	0	0	0	0	0	940	340	0	0	0	90	0	0	0	0	0	0	0	0	0	2	**1,763**	**100.0**
	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	0	**0**	**0.0**
	Total	**110**	**281**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**940**	**340**	**0**	**0**	**0**	**90**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**1,763**	**100.0**
Rabbits	No	0	24	0	0	0	0	0	0	0	117	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	**166**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**24**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**117**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**25**	**0**	**166**	**100.0**
Pigs	No	0	0	0	0	0	18	0	0	0	0	39	0	0	0	26	0	0	0	0	105	0	0	0	0	0	**188**	**85.5**
	Yes	0	0	0	0	0	1	0	0	0	0	0	0	0	0	31	0	0	0	0	0	0	0	0	0	0	**32**	**14.5**
	Total	**0**	**0**	**0**	**0**	**0**	**19**	**0**	**0**	**0**	**0**	**39**	**0**	**0**	**0**	**57**	**0**	**0**	**0**	**0**	**105**	**0**	**0**	**0**	**0**	**0**	**220**	**100.0**
Goats	No	0	70	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**87**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**70**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**17**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**87**	**100.0**
Sheep	No	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**9**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**100.0**
Marmoset and tamarins	No	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**11**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**100.0**
Rhesus monkey	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0.0**
	Yes	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	**14**	**100.0**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**14**	**100.0**
Domestic fowl	No	0	0	0	0	0	189	0	0	0	0	601	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**790**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**0**	**0**	**0**	**0**	**189**	**0**	**0**	**0**	**0**	**601**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**790**	**100.0**
Xenopus	No	4,998	94	0	0	0	0	0	0	0	0	423	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**5,515**	**100.0**
	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	0	**0**	**0.0**
	Total	**4,998**	**94**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**423**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5,515**	**100.0**
Other amphibians	No	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**33**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**33**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**33**	**100.0**
Zebra fish	No	2,694	4,293	0	0	0	119	2,310	0	628	5,863	33,986	0	0	2,059	648	0	602	0	521	20	0	0	0	3,836	11,361	**68,940**	**96.2**
	Yes	0	0	0	76	0	0	0	0	0	0	0	0	267	0	0	0	0	0	335	0	2,037	0	0	0	0	**2,715**	**3.8**
	Total	**2,694**	**4,293**	**0**	**76**	**0**	**119**	**2,310**	**0**	**628**	**5,863**	**33,986**	**0**	**267**	**2,059**	**648**	**0**	**602**	**0**	**856**	**20**	**2,037**	**0**	**0**	**3,836**	**11,361**	**71,655**	**100.0**
Salmon, trout, chars and graylings	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1,749	0	0	0	0	0	0	**1,749**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1,749**	**0**	**0**	**0**	**0**	**0**	**0**	**1,749**	**100.0**
Other fish	No	0	0	0	0	0	0	0	0	0	0	912	0	0	0	0	0	0	0	200	0	44	0	0	736	0	**1,892**	**100.0**
	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	0	**0**	**0.0**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**912**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**200**	**0**	**44**	**0**	**0**	**736**	**0**	**1,892**	**100.0**
All Species	**No**	**15,478**	**23,663**	**0**	**0**	**0**	**5,218**	**5,060**	**0**	**4,161**	**47,573**	**158,365**	**7,694**	**582**	**2,262**	**3,564**	**46**	**602**	**7,520**	**5,235**	**829**	**1,282**	**0**	**0**	**21,433**	**16,846**	**327,413**	**99.1**
All Species	**Yes**	**0**	**1**	**0**	**76**	**0**	**1**	**0**	**0**	**0**	**0**	**102**	**20**	**281**	**0**	**177**	**0**	**0**	**0**	**335**	**0**	**2,037**	**0**	**0**	**0**	**15**	**3,045**	**0.9**
All Species	**Total**	**15,478**	**23,664**	**0**	**76**	**0**	**5,219**	**5,060**	**0**	**4,161**	**47,573**	**158,467**	**7,714**	**863**	**2,262**	**3,741**	**46**	**602**	**7,520**	**5,570**	**829**	**3,319**	**0**	**0**	**21,433**	**16,861**	**330,458**	**100.0**

Table 3.2: Uses of animals for the maintenance of colonies of established genetically altered animal lines by species, reuse and Member State

	Reuse	Austria	Belgium	Croatia	Czechia	Denmark	Estonia	Finland	France	Germany	Greece	Ireland	Italy	Luxembourg	Netherlands	Norway	Poland	Portugal	Romania	Slovakia	Spain	Sweden	Total	%
Mice	No	6,203	24,256	1,245	0	2,994	0	572	470,114	76,631	20	279	1,190	167	3,619	4,055	0	3,391	69	358	22,520	1,865	**619,548**	**99.9**
	Yes	0	366	0	0	0	0	0	0	0	0	0	0	0	0	0	268	0	0	0	0	0	**634**	**0.1**
	Total	**6,203**	**24,622**	**1,245**	**0**	**2,994**	**0**	**572**	**470,114**	**76,631**	**20**	**279**	**1,190**	**167**	**3,619**	**4,055**	**268**	**3,391**	**69**	**358**	**22,520**	**1,865**	**620,182**	**100.0**
Rats	No	0	0	0	0	0	0	4	14,469	1,848	0	0	0	0	0	0	0	18	0	191	11	0	**16,541**	**99.9**
	Yes	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**16**	**0.1**
	Total	**0**	**16**	**0**	**0**	**0**	**0**	**4**	**14,469**	**1,848**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**18**	**0**	**191**	**11**	**0**	**16,557**	**100.0**
Dogs	No	0	0	0	0	0	0	0	27	0	0	0	0	0	0	0	0	0	0	0	0	0	**27**	**100.0**
	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**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**27**	**100.0**
Zebra fish	No	0	0	0	0	0	0	0	7,168	6,074	0	229	57	175	137	5,315	0	815	0	0	1,380	0	**21,350**	**72.5**
	Yes	0	255	0	0	0	0	0	0	21	0	0	0	0	0	7,815	0	0	0	0	0	0	**8,091**	**27.5**
	Total	**0**	**255**	**0**	**0**	**0**	**0**	**0**	**7,168**	**6,095**	**0**	**229**	**57**	**175**	**137**	**13,130**	**0**	**815**	**0**	**0**	**1,380**	**0**	**29,441**	**100.0**
Other fish	No	0	0	0	0	0	0	0	0	348	0	0	0	0	0	0	0	0	0	0	0	0	**348**	**100.0**
	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**
	Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**348**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**348**	**100.0**
All Species	**No**	**6,203**	**24,256**	**1,245**	**0**	**2,994**	**0**	**576**	**491,778**	**84,901**	**20**	**508**	**1,247**	**342**	**3,756**	**9,370**	**0**	**4,224**	**69**	**549**	**23,911**	**1,865**	**657,814**	**98.7**
	Yes	**0**	**637**	**0**	**0**	**0**	**0**	**0**	**0**	**21**	**0**	**0**	**0**	**0**	**0**	**7,815**	**268**	**0**	**0**	**0**	**0**	**0**	**8,741**	**1.3**
	Total	**6,203**	**24,893**	**1,245**	**0**	**2,994**	**0**	**576**	**491,778**	**84,922**	**20**	**508**	**1,247**	**342**	**3,756**	**17,185**	**268**	**4,224**	**69**	**549**	**23,911**	**1,865**	**666,555**	**100.0**