

MEMBER STATE NARRATIVE

Member State: Belgium

Year: 2023

(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-authorized 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-authorized or not: /
- Details of the use: /
- Reasons why the ‘severe’ classification was exceeded: /