

MEMBER STATE NARRATIVE

Member State: Belgium

Year: 2022

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

The total number of animal procedures conducted in 2022 encompass:

- all uses of animals for research, testing, routine production, education, and training purposes (433495);
- all uses of animals for the creation of new genetically altered animal lines (23732) and
- all uses of animals for the maintenance of genetically altered animal lines (16102).

In 2022, there were 473329 animal procedures conducted, which marked a slight decrease compared to the 477675 procedures performed in 2021. This reduction amounted to 4346 fewer animal procedures, accounting for an approximate decline of 0,91%.

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

In this category, there was a slight decrease, with 434327 uses in 2021 and 433495 in 2022.

When looking at the species used for research, testing, routine production, education, and training, certain trends emerge. Procedures involving mice increased by around 5,9% from 2021 to 2022, while the use of rabbits showed a modest increase of around 2,9%. The use of domestic fowl shows a significant decrease of roughly 24,3%. This decrease can be partly explained by a reduction in the number of feed additive studies. In such studies, the primary parameter is often feed conversion, which can be evaluated at group level and requires multiple replicates of several animals to provide sufficient statistical power. The number of Zebra fish procedures decreased by around 4,7% and the number of rat procedures remained relatively stable with a slight decrease of about 0,4%. Less commonly used species show more pronounced variations. However, due to the small sample sizes, these fluctuations are not relevant when considering general trends.

Looking at the reuse of animals used for research, testing, routine production, education, and training, we see that the number of reuses remains stable. In 2021, the reuse rate was 0,68%, and in 2022, it was 0,65%.

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

There is a decrease in the use of animals for the creation of new genetically altered lines (-17%). This decrease is mainly due to a decrease in the number of animals used to establish new lines in basic research on the immune system (2021: 5801; 2022: 680), on the nervous system (2021: 7048; 2022: 5541) and oncology (2021: 7746; 2022: 5920). This does not seem to be related to the total number of animals used in these fields. There is, in fact, an increase in the use of animals for basic research on the nervous system and in oncology. However, there is a decrease in studies related to the immune system.

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

of about 16% from 23790 in 2021 to 19980 in 2022. This is related to the above-mentioned decrease in the number of animals used to establish new lines in basic research on the immune system, the nervous system, and oncology. Conversely, the number of procedures on rats increased from 133 in 2021 to 180 in 2022, an increase of about 35%. Of course, this involves a much smaller absolute number. In addition, goats were introduced into this category in 2022, with 32 procedures, marking a new trend in their use for genetic research. This occurred in an applied study in the field of Animal Diseases and Disorders. *Xenopus*, which had no recorded procedures since 2017, was reintroduced in 2022 with 84 procedures, indicating a new use for the creation genetically altered animal lines. This involves a specific user working with modified *Xenopus* lines in the field of basic research on the nervous system and basic research on oncology. Finally, Zebra fish shows a decrease in procedures of around 23%, from 4395 in 2021 to 3336 in 2022. This decrease is distributed across different domains where animals have been used to create genetically modified lines.

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

In this category, use increased from 14760 uses in 2021 to 16102 uses in 2022. This represents an increase of about 9,08%.

Looking at the use of animals for the maintenance of genetically altered animal lines in 2022, we see that the number of procedures on mice increased slightly from 14041 in 2021 to 15906 in 2022, an increase of about 13,2%. In contrast, the number of procedures on rats decreased significantly, from 719 in 2021 to only 48 in 2022, indicating a significant decline. The use of Zebra fish, which had 120 procedures in 2020 and 0 procedures in 2021, saw an increase to 148 procedures in 2022.

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

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

In particular, there was a significant increase in the number of uses in oncology. For example, there was an increase of 50,31% in 2022 (61834 uses) compared to 2019 (41138 uses). The main areas of research in the basic research category in 2022 were oncology (32,68%), immune system (17,77%) and nervous system (15,36%).

A decrease of 12,23% was observed in the 'Translational and applied research' category in 2022 (135034 procedures) compared to 2021 (153838 procedures). The temporary increase in the number of procedures in this category in 2021 (from 120195 in 2020 to 153838 in 2021, an increase of 28%) has now been reversed. In terms of the number of uses, it is back to a level comparable to 2019 (130712 uses). Between 2021 and 2022, the largest decrease was observed in the categories of 'Animal diseases and disorders' (-11863 uses, i.e. a decrease of 25%). This is due to several factors. On the one hand, there is a decrease in the number of studies on feed additives, as mentioned above. On the other hand, efforts have been made to refine experimental designs. For example, there has been a strong focus on better characterisation of pathogenic strains, including the validation of different animal models. This allows the desired effect to be assessed with a smaller number of animals.

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

The category 'Training for the acquisition, maintenance or improvement of vocational skills' increased by 40,69% in 2022 compared to 2021. Three-quarters of the animals used in this category were rodents but 15% were birds (domestic fowl and turkeys) and 10% were pigs. The use of domestic fowl and turkeys involves inducing certain conditions in the animals in order to educate and train poultry veterinarians and professionals working in the field to recognise injuries caused by these conditions and to intervene promptly. In the case of pigs, the main aim is to practise various surgical techniques using robotic surgery. Training on live animals is the final training step required to closely mimic working on live humans.

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

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

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

Despite what appeared to be a trend within the mild (downward trend) and moderate (upward trend) categories in recent years, this does not continue in 2022. Instead, in 2022 there is an increase of 8,9% within the mild category and a decrease of 10,07% within the moderate category.

We observe a significant increase in the mild category in 'Basic research' (from 88193 mild uses in 2021 to 99435 mild uses in 2022) and in the 'Regulatory use and routine production' category (from 68593 mild uses in 2021 to 92207 mild uses in 2022). The latter is partly explained by a shift in severity from moderate to mild within the 'Blood products' category.

For moderate procedures, there is an increase in 'Basic research' (from 67632 moderate uses in 2021 to 72461 moderate uses in 2022). As mentioned above, there is a decrease in the number of moderate uses in the 'Regulatory use and routine production' category. There was also a decrease in the number of moderate uses in the 'Translational and applied research' category (from 71502 moderate uses in 2021 to 52507 moderate uses in 2022). This is related to the overall decrease in the 'Translational and applied research' category in 2022.

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

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

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

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

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

With regard to the use of fish, the category 'Other fish' accounts for 17,05% in 2022. Within this category, it consists mainly of *Oreochromis niloticus* (3351 uses, or 39%), *Rutilus rutilus* (1290 uses, or 15%) and *Anguilla anguilla* (1216 uses, or 14%). These species are only used in the category of basic research, of ethology, animal behaviour and animal biology and for species preservation.

Regarding the use of amphibians, the use of 'Other amphibians' accounts for 20,22% in 2022. In this category, it is mainly the use of *Lithobates catesbeianus* (70 uses, or 93,33%) for the protection of the natural environment in the interest of human or animal health or welfare.

At the purpose level, within the category 'Regulatory uses – quality control', the category 'Other quality control' accounts for 14,41% (or 4420 uses) of the total. These 4420 mice were used for EPO potency determination. More mice were used for this purpose in recent years compared to 2019 due to the need to validate new standards for erythropoietin. In addition, there was a higher use in 2022 due to a shortage of EPO raw material in 2021, which required more batches to be tested to make up the shortfall.

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

169 animals were used in the category 'Monoclonal and polyclonal antibodies (excluding ascites)', including 115 mice, 53 rats and 1 *Llama Glama*. In vitro methods for the production of monoclonal antibodies have been validated by the EURL ECVAM but animals are still used for their production in Belgium. In general, users prefer in vitro methods such as the phage display method, but sometimes it appears that synthetic monoclonal antibodies are slightly different from animal antibodies and are more difficult to produce. Animals are still used for the production of polyclonal antibodies and no alternative method has been validated. Users report that it would be possible to produce polyclonal antibodies in vitro by mixing monoclonal antibodies,

but these mixtures never fully reproduce the performance of the in vivo polyclonal antibody, especially for complex antigens (e.g. cells). In addition, some products require marketing authorisation and cannot be easily changed. These products are used to make a life-saving drug for which there is currently no alternative.

(g) Details on cases where the 'severe' classification is exceeded, whether pre-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: /