HPRA
An tÚdarás Rialála Táirgí Sláinte
Health Products Regulatory Authority

Annual statistical report for animals used in Ireland under scientific animal protection legislation - 2021

16 DECEMBER 2022

CONTENTS

1 INTRODUCTION

The Health Products Regulatory Authority (HPRA) is the State agency with responsibility for the protection of animals used for scientific purposes, the regulation of human and veterinary clinical trials, and the regulation of human and veterinary medicines, medical devices and other health products, amongst other regulatory functions. From 1 January 2013, an EU Directive[1] to protect animals used for scientific purposes came into effect in Ireland. In January 2013, the HPRA became the competent authority responsible for the Directive's implementation.

The Directive is among the world's most advanced pieces of legislation concerning animal welfare. The restrictions and standards set by the Directive aim to enhance animal welfare and ensure that animals are used in studies only when their use is absolutely necessary and following independent assessment. The Directive firmly anchors in EU Legislation the 3R principles, i.e., Replacement, Reduction, and Refinement:

- **Replacement** involves the acceleration of the development and use of models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals. Examples of alternative methods would include *in vitro* tests such as the use of cell lines, computer and mathematical simulation and modelling, video material, or the use of invertebrates such as fruit flies or worms.

- **Reduction** refers to methods that minimise the number of animals used per project or study consistent with the scientific aims. It is essential that studies using animals are appropriately designed and analysed to ensure robust and reproducible findings. Reduction also includes methods that allow the information gathered per animal in a study to be maximised in order to reduce the use of additional animals. Examples of this include the use of some imaging modalities which allow repeated measurements in the same animal to be taken over time (rather than, for example, imaging different animals at each time point) or microsampling of blood, where limiting the amount of blood taken each time to small volumes enables repeat sampling in the same animal.

- **Refinement** refers to methods that minimise the pain, suffering, distress or lasting harm that may be experienced by the animals, and which improve their welfare. Refinement means that animals are provided with the best possible care and this applies to all aspects of animal use, from the animals' housing and husbandry to the scientific procedures performed on them. Examples of refinement include ensuring that animals are provided with housing that allows the expression of species-specific behaviours, using appropriate anaesthesia and analgesia to minimise pain, and training animals to cooperate with procedures to minimise any stress.

Although complete replacement of animal studies is the ultimate goal of the Directive, for the moment the use of live animals continues to be necessary to protect human and animal health and the environment. Where biological processes are not sufficiently understood or are very complex, non-animal research or test methods are often not available. After generating as much information as possible using non-animal alternatives, animal studies can be necessary to fill knowledge gaps. However, the Directive plays a vital role in protecting and improving the welfare of those animals that are required to be used for scientific purposes.

[1] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes

The HPRA regulates the sector by means of authorisation at three levels:

1. **Breeder/supplier/user establishments:** Breeders and suppliers of animals, as well as establishments where procedures are performed, must be authorised and are subject to HPRA inspections. During 2021, there were 29 inspections completed to monitor animal welfare standards and compliance with legislation, with 41% of these performed as unannounced inspections.

2. **Projects:** Scientific procedures involving animals can only take place following a detailed submission of the planned study/studies and subsequent approval by the HPRA on the basis of a favourable harm/benefit analysis.

3. **Individuals:** Any person wishing to carry out scientific procedures involving animals, as well as project managers and those conducting euthanasia in an authorised establishment, must be adequately trained to do so and hold a HPRA individual authorisation.

The HPRA aims to improve the welfare of animals used for scientific purposes and to promote the principles of the 3Rs. Every application received for a project involving animals is subject to a detailed evaluation process rooted in the 3R principles and requires robust scientific justification for the research techniques being applied. The HPRA considers whether alternative (non-animal) methods are available or appropriate, as alternatives to the use of live animals must be used where possible. Where non-animal methods to address the specific research question are not available/appropriate, the likely impact on the animals must be minimised in so far as possible by applying all appropriate refinements, and any harms experienced by the animals must be outweighed by the expected benefits of the work. In fulfilment of the HPRA's mandate to promote the 3Rs, in 2021 the HPRA continued its ongoing efforts to enhance awareness and utilisation of non-animal alternatives. The HPRA continued to promote the application of the principles of Replacement, Reduction, and Refinement in the conduct of scientific studies in animals through its work, including the regular dissemination of pertinent information to the regulated sector.

The objective of this report is to present statistical data on the number of uses of animals for scientific purposes in Ireland during 2021 in accordance with Article 54(2) of the Directive. This is the ninth report to be prepared by the HPRA since it became the competent authority for the protection of animals used for scientific purposes.

As in previous years, the data provided are based on self-declarations by the establishments concerned. The data are presented in this report in accordance with the Commission Implementing Decision 2020/569/EU, which sets out a common format for the submission of information to the European Commission. In line with the EU summary reports under Directive 2010/63/EU on the uses of animals for scientific purposes within the EU and Norway, data has been categorised in this report as follows:

- number of animals used in research, testing, routine production, and education and training purposes[2]
- details of all uses (first and any subsequent reuse) of animals for research and testing
- numbers and uses of animals for the creation and maintenance of genetically altered animal lines

[2] Hereafter referred to as 'research and testing'

Please refer to the Appendix for definitions relating to some of the terminology used in this report.

2 SUMMARY

In 2021, there were a total of 122,383 uses of animals in procedures for research and testing purposes, with reuse representing <1% of this number (825 uses). Some 121,558 animals were reported as being used for the first time for research and testing purposes in 2021. Mice were the most commonly used species at 75% of total animal use. In addition, 1,863 mice were reported as having been used to create and maintain colonies of genetically altered animals. These 1,863 mice are not considered by the European Commission to have been directly used in research and testing.

Of the total number of uses of animals in procedures for research and testing purposes, 67% were used for regulatory purposes, which refers to legal requirements to test the safety, quality, and potency of medicines (e.g., biological medicines such as vaccines). Of the total number of uses of animals in procedures for research and testing purposes, 7,413 involved genetically altered animals, which represents 6% of all animal use.

The most frequently reported actual severity experienced by animals during their uses in procedures for research and testing purposes was mild at 58%, followed by moderate at 26%. Of the animals reported as being used for the creation and maintenance of genetically altered lines, 98% were reported as having experienced an actual severity of mild.

3 DATA ON THE USES OF ANIMALS FOR RESEARCH AND TESTING PURPOSES

3.1 Species and numbers of uses of animals for research and testing purposes

Table 1 shows the number of animals used (by species) for the first time in procedures performed for research and testing purposes. Each use of an animal extends from the time when the first intervention is applied to the animal until the completion of data collection under a project, or when the animal is removed from the project.

Mice (75% of total first uses of animals) were the most commonly used species. The second most commonly used species was rats, followed by fish. The animals reported as being used under the category 'other rodents' (<1%) were red squirrels being studied as part of a conservation project aiming to protect their population numbers and habitats. The category 'other fish' (<1%) represents wild fish species such as Bluefin tuna and Blue sharks being studied for conservation projects where their movements are closely monitored as part of efforts to improve the survival of these species. Likewise, the category 'other birds' (<1%) represents wild bird species (European storm petrel and great tits) being studied in monitoring, conservation, and ecology projects.

It should be noted that the following species have been excluded from this table as they were not reported as being used for the first time for research and testing purposes in 2021; hamsters (Chinese), Mongolian gerbils, other carnivores, other mammals, turkeys, reptiles, rana, other amphibians, zebrafish, sea bass, guppy, swordtail, molly, platy, cephalopods and non-human primates.

Table 1: Numbers animals used for the first time by species

Animal species	Number of uses	Percentage
Mice	91,497	75.27%
Rats	17,050	14.03%
Guinea pigs	774	0.64%
Hamsters (Syrian)	32	0.03%
Other rodents	19	0.02%
Rabbits	657	0.54%
Cats	13	0.01%
Dogs	83	0.07%
Ferrets	358	0.29%
Horses, donkeys and cross-breeds	9	0.01%
Pigs	727	0.60%
Goats	31	0.03%
Sheep	2,806	2.31%
Cattle	2,108	1.73%
Domestic fowl	98	0.08%
Other birds	92	0.08%
Xenopus	53	0.04%
Salmon, trout, chars and graylings	4,986	4.10%
Other fish	93	0.08%
Total uses	**121,558**	**100.00%**

3.2 Reuse of animals

Table 2 shows the proportion of reuse (see Appendix for definition), which represents 0.67% of total animal use. Under the legislation, animals are only permitted to be reused on second or subsequent projects if the severity they have experienced to date is mild or moderate (see Appendix for definition of severity categories) and they have been assessed as having returned to full general health between projects.

It should be noted that the true number of animals that are reused cannot be deduced from this data due to the fact that some animals may be reused more than once (i.e., the figure 825 represents *uses of animals,* not actual numbers of animals reused). Cattle, sheep, horses, dogs, and cats were the species reused during 2021. The reuse of cattle was in agricultural research studies investigating, for example, ways to protect the environment through the reduction of methane emissions. Sheep were reused in a study investigating ovine parasitic disease. The horses that were reused were used for the purposes of training veterinary students, and the severity of the procedures did not exceed mild. In Ireland, cats and dogs are used exclusively for research into animal diseases and disorders, and the development and testing of veterinary medicines for companion animals.

Table 2 shows the total numbers of all uses of animals (first time and reuse) in procedures performed for research and testing purposes, broken down by species.

Table 2: Numbers animals used (first time and reuse) by species

Animal species	First uses	Reuses	Percentage of reuses	Total uses (first use + reuse)
Mice	91,497	0	0.00%	91,497
Rats	17,050	0	0.00%	17,050
Guinea pigs	774	0	0.00%	774
Hamsters (Syrian)	32	0	0.00%	32
Other rodents	19	0	0.00%	19
Rabbits	657	0	0.00%	657
Cats	13	52	80.00%	65
Dogs	83	111	57.22%	194
Ferrets	358	0	0.00%	358
Horses, donkeys and cross-breeds	9	10	52.63%	19
Pigs	727	0	0.00%	727
Goats	31	0	0.00%	31
Sheep	2,806	3	0.11%	2,809
Cattle	2,180	649	22.94%	2,829
Domestic fowl	98	0	0.00%	98
Other birds	92	0	0.00%	92
Xenopus	53	0	0.00%	53
Salmon, trout, chars and graylings	4,986	0	0.00%	4,986
Other fish	93	0	0.00%	93
Total uses	**121,558**	**825**	**0.67%**	**122,383**

3.3 Origin of animals at the first use

Table 3 shows the number of animals according to their place of birth, but only includes animals used for the first time as the place of birth is not recorded for animals on their second (or subsequent) use(s). The majority (88%) of animals were born in the EU at a registered breeder, which means that they were born at breeding establishments authorised under the Directive. Animals born in the EU but not at a registered breeder (7%) includes wild animals and farm animals. Animals born in the rest of Europe (5%) represents rats and mice sourced from establishments authorised in the UK (since the UK is no longer an EU Member State). Animals born in the rest of the world (<1%) represents animals that have been obtained from breeding establishments outside of Europe (e.g. specific strains of mice not available in Europe) and wild fish species that have migrated into Irish waters from other regions.

Table 3: Origin of animals at the first use

Place of Birth	Number of uses	Percentage
Animals born in the EU at a registered breeder	106,805	87.86%
Animals born in the EU not at a registered breeder	8,316	6.84%
Animals born in rest of Europe	6,346	5.22%
Animals born in rest of world	91	0.07%
Total uses	**121,558**	**100.00%**

3.4 Project purposes

Table 4 shows the primary purposes for which animals were used in research and testing. The most common primary purpose at 67% was 'Regulatory use and routine production'. This is defined as the 'use of animals in procedures with a view to satisfying legal requirements for producing, placing and maintaining products/substances on the market, including safety and risk assessment for food and feed'.

The primary purposes are further sub-divided in Tables 5-11. It should be noted that the primary purposes 'Higher education' and 'Forensic enquiries' are excluded from this table as no uses were reported under these purposes for 2021.

Table 4: Primary purpose for which animals are used

Primary purpose	Number of uses	Percentage
Basic research	13,445	10.99%
Translational and applied research	19,875	16.24%
Regulatory use and routine production	82,325	67.27%
Protection of the natural environment in the interests of the health or welfare of human beings or animals	6,314	5.16%
Preservation of the species	217	0.18%
Training for the acquisition, maintenance or improvement of vocational skills	207	0.17%
Total uses	**122,383**	**100.00%**

Table 5 shows the number of uses of animals in more specific categories of 'Basic research'. 'Basic research' refers to studies of a fundamental nature, which are designed to add knowledge about the structure, functioning, or behaviour of organisms. It should be noted that the sub-fields of 'urogenital/reproductive system', 'endocrine system/metabolism', 'developmental biology' and 'other basic research' were excluded from this table as no uses under these sub-fields were reported for 2021.

The most common sub-field of 'Basic research' at 42% was 'immune system', which relates to research investigating the functioning and activities of the immune system in health and disease. The next most common sub-field at 33% was 'nervous system'. Nervous system research involves studies that investigate how the brain, spinal cord, and nerves function in both health and in disease (e.g., Alzheimer's disease or Parkinson's disease). These types of studies most commonly use mice and rats.

The third most common sub-field was 'ethology/animal behaviour/animal biology', representing 15% of all basic research performed. In Ireland, this is mainly accounted for by agricultural research into farm animal biology, or research into the behaviour of wild animals (e.g., fish or bird tracking studies).

Table 5: Uses of animals for basic research

Basic Research	Number of uses	Percentage
Oncology	20	0.15%
Cardiovascular, blood and lymphatic system	31	0.23%
Nervous system	4,387	32.63%
Respiratory system	31	0.23%
Gastrointestinal system including liver	118	0.88%
Musculoskeletal system	3	0.02%
Immune system	5,674	42.20%
Sensory organs (skin, eyes and ears)	431	3.21%
Multisystemic	728	5.41%
Ethology / animal behaviour /animal biology	2,022	15.04%
Total uses	**13,445**	**100.00%**

Table 6 shows the number of uses of animals in more specific categories of 'Translational and applied research', which refers to studies which aim to prevent, diagnose, detect or treat disease in animals or humans, as well as studies which aim to improve animal nutrition and welfare. It should be noted that the sub-fields of 'diagnosis of diseases', 'plant diseases' and 'non-regulatory toxicology and ecotoxicology' were excluded from this table as no uses under these sub-fields were reported for 2021.

The most common sub-field of 'Translational and applied research' at 40% was research into 'human nervous and mental disorders'. This includes research into diagnostics and treatments for diseases such as epilepsy, autism, and depression, using mice and rats. The next most common sub-field at 12% was research into 'human immune disorders'. This includes research into conditions that have an immune component such as multiple sclerosis and rheumatoid arthritis. The third most common sub-field was 'animal diseases and disorders'. Studies conducted under this project purpose sub-field in 2021 included research into non-antibiotic alternative treatments for preventing infection in piglets, pneumonia in calves, and the investigation of new treatments for parasitic infections in dogs.

Table 6: Uses of animals for translational and applied research

Translational and applied research	Number of uses	Percentage
Human cancer	533	2.68%
Human infectious disorders	550	2.77%
Human cardiovascular disorders	890	4.48%
Human nervous and mental disorders	8,042	40.46%
Human respiratory disorders	723	3.64%
Human gastrointestinal disorders including liver	1,045	5.26%
Human musculoskeletal disorders	1,718	8.64%
Human immune disorders	2,387	12.01%

Translational and applied research	Number of uses	Percentage
Human urogenital/reproductive disorders	80	0.41%
Human sensory organ disorders (skin, eyes and ears)	470	2.36%
Human endocrine/metabolism disorders	278	1.40%
Other human disorders	137	0.69%
Animal diseases and disorders	1,991	10.02%
Animal nutrition	628	3.16%
Animal welfare	403	2.03%
Total uses	**19,875**	**100.00%**

Table 7 shows the breakdown of animal uses for 'Regulatory use and Routine production'. The majority of reported uses (>99%) can be attributed to quality control testing. Routine production refers to the use of animals for the production of biological material.

Table 7: Uses of animals for regulatory testing

Regulatory use	Number of uses	Percentage
Quality control (including batch safety and potency testing)	82,227	99.88%
Other efficacy and tolerance testing	18	0.02%
Toxicity and other safety testing including pharmacology	36	0.04%
Routine production by product type	44	0.05%
Total uses	**82,325**	**100.00%**

Table 8 shows a further breakdown of animal use for 'Quality control' tests. Quality control refers to animals used in the testing of purity, stability, efficacy, and potency parameters of a final medicinal product, in order to satisfy regulatory requirements. The majority of quality control tests were for batch potency (86%), and of these tests, 81% were performed on mice. It should be noted that the sub-field of 'other quality controls' was excluded from this table as no uses under this sub-field were reported for 2021.

Batch potency testing is required for biological products, which are products made in a living system such as a microorganism, or plant or animal cells, as opposed to being manufactured through chemical synthesis. The nature of biological products is that they can be inherently variable, and therefore it is necessary to perform batch potency testing to ensure that each manufactured batch of product is of a consistent strength, and therefore both safe and effective for patients. It is also required as a safety/efficacy test for certain types of vaccine. In relation to pyrogenicity testing (<1%), non-animal alternatives have been developed to replace this type of testing for certain medicines. However, there are still some limited circumstances in which it is not possible to use a non-animal alternative to test for pyrogenicity (e.g., for some blood and protein-based human medicines), and therefore currently it is necessary to continue to employ animal-based tests in some instances.

Table 8: Uses of animals for regulatory testing - quality control

Quality control	Number of uses	Percentage
Batch safety testing	10,674	12.98%
Pyrogenicity testing	657	0.80%
Batch potency testing	70,896	86.22%
Total uses	**82,227**	**100.00%**

Table 9 relates to the use of animals for regulatory testing under the sub-field 'Toxicity and other safety testing including pharmacology'. Animals reported under this sub-field in 2021 were used to test the safety of medical devices (pigs) and the kinetics of new veterinary medicinal products (dogs). Since there were no animals reported under any other sub-fields of 'Toxicity and other safety testing including pharmacology' in 2021 these have been excluded from this table.

Table 9: Uses of animals for regulatory testing - toxicity and other safety testing including pharmacology

Toxicity and other safety testing including pharmacology	Number of uses	Percentage
Kinetics	32	88.89%
Other toxicity testing	4	11.11%
Total uses	**36**	**100.00%**

Table 10 shows a further breakdown of animal use for 'Routine production uses by product type'. Animals reported under this category were all used for the production of blood-based products. This refers to e.g., the collection of animal blood for use in other regulatory tests required under legislation for human medicinal products, as well as for use in diagnostic tests for diseases of cattle. The sub-fields 'monoclonal antibody by mouse ascites method', 'monoclonal and polyclonal antibodies (excluding ascites method); and 'other products' were excluded from this table as no uses under these sub-fields were reported in 2021.

Table 10: Routine production uses by product type

Product type	Number of uses	Percentage
Blood based products	44	100.00%
Total uses	**44**	**100.00%**

3.5 Use of animals to meet legislative requirements

Table 11 shows which type of legislation is being satisfied in the performance of the regulatory tests for which animals were used. Only categories of legislation under which animal use was reported are included in this table. The vast majority (>99%) were performed on medicinal products manufactured for use in humans. <1% of uses were to satisfy legislation on medicinal products for veterinary use and their residues. Testing to satisfy medical devices legislation is required to place medical devices on the market.

Table 11: Regulatory testing by type of legislation

Testing by Legislation	Number of uses	Percentage
Legislation on medicinal products for human use	82,227	99.93%
Legislation on medicinal products for veterinary use and their residues	50	0.06%
Medical devices legislation	4	0.00%
Total uses	**82,281**	**100.00%**

Table 12 shows the geographical origin of the legal requirement. All tests were performed to satisfy EU legislative requirements.

Table 12: Origin of legislative requirement

Legislative Requirement	Number of uses	Percentage
Legislation satisfying EU requirements	82,281	100.00%
Legislation satisfying national requirements only [within EU]	0	0.00%
Legislation satisfying non-EU requirements only	0	0.00%
Total uses	**82,281**	**100.00%**

3.6 Use by genetic status for research and testing purposes

Table 13 shows the number of uses of animals for research and testing purposes that had a genetic alteration, broken down by whether the animal exhibited a harmful phenotype at the time of the study or not. The majority of animals (94%) were not genetically altered, with 2% of all uses involving animals with harmful phenotypes (see Appendix for definitions).

Table 13: Genetic status

Genetic Status	Number of uses	Percentage
Not genetically altered	114,970	93.94%
Genetically altered without a harmful phenotype	4,485	3.66%
Genetically altered with a harmful phenotype	2,928	2.39%
Total uses	**122,383**	**100.00%**

3.7 Actual severity of uses of animals for research and testing purposes

Table 14 shows the reported actual severity experienced by the animals during their uses for research and testing purposes. Overall, <2% of uses were classified as non-recovery, 58% were classified as mild, 26% were moderate and 14% were severe (see Appendix for definitions). Of the animals that were reported as experiencing severe severity in 2021, >99% were mice. It should be noted that there were no reports received of the severe classification being exceeded, nor did the HPRA grant any exemptions for the severe classification to be exceeded.

Table 14: Classification of actual severity

Severity	Number of uses	Percentage
Non-recovery	1,932	1.58%
Mild [up to and including]	71,286	58.25%
Moderate	32,001	26.15%
Severe	17,164	14.02%
Total uses	**122,383**	**100.00%**

4 DATA ON THE USES OF ANIMALS FOR THE CREATION AND MAINTENANCE OF GENETICALLY ALTERED ANIMAL LINES

Table 15 shows the number of animals (by species) used for the creation of new genetically altered animal lines for the first time. There was no reuse of animals reported for the creation of new genetically altered animal lines in 2021.

Table 15: Number of animals (by species) used for the creation of new genetically altered animal lines for the first time

Animal species	Number of uses	Percentage
Mice	60	13.30%
Zebrafish	391	86.70%
Total uses	**451**	**100.00%**

Table 16 shows the uses of animals for the creation of new genetically altered lines by severity. 100% of animals used in 2021 for the creation of a new genetically altered line were reported as having experienced mild severity.

Table 16: Uses of animals for the creation of new genetically altered animal lines by severity

Severity	Number of uses	Percentage
Non-recovery	0	0.00%
Mild [up to and including]	451	100.00%
Moderate	0	0.00%
Severe	0	0.00%
Total uses	**451**	**100.00%**

Table 17 shows the number of animals used for the creation of new genetically altered lines by genetic status of the animals. 13% of animals used in 2021 for the creation of a new genetically altered line were reported as genetically altered without a harmful phenotype, whereas 87% were reported as genetically altered with a harmful phenotype.

Table 17: Uses of animals for the creation of new genetically altered animal lines by genetic status

Genetic status	Number of uses	Percentage
Not genetically altered	0	0.00%
Genetically altered without a harmful phenotype	60	13.30%
Genetically altered with a harmful phenotype	391	86.70%
Total uses	**451**	**100.00%**

Table 18 shows the uses of animals for the creation of new genetically altered lines by type of 'Basic research' purposes. 100% of animals used for the creation of a new genetically altered line were reported under the 'Basic research' sub-field 'nervous system'. Since the remaining 'Basic research' purposes had no animals reported, they are excluded from this table.

Table 18: Uses of animals for the creation of new genetically altered animal lines by type of basic research purposes

Basic research	Number of uses	Percentage
Nervous system	60	100.00%
Total uses	**60**	**100.00%**

Table 19 shows the uses of animals for the creation of new genetically altered lines by type of 'Translational and applied research' purposes. The only 'Translational and applied research' sub-field under which animals were reported as being used for the creation of a new genetically altered line was 'human sensory organ disorders (skin, eyes and ears)'. Since the remaining 'Translational and applied research' sub-fields had no animals reported, they are excluded from this table.

Table 19: Uses of animals for the creation of new genetically altered animal lines by type of translational and applied research purposes

Translational and applied research	Number of uses	Percentage
Human sensory organ disorders (skin, eyes and ears)	391	100.00%
Total uses	**391**	**100.00%**

Table 20 shows the number of animals used (by species) for the maintenance of established genetically altered lines for the first time. Mice and zebrafish were the only species of animal reported as being used for the maintenance of established genetically altered lines in 2021 and therefore other species have been excluded from this table. There was no reuse of animals reported for the maintenance of established genetically altered lines in 2021.

Table 20: Number of animals used for the maintenance of established genetically altered animal lines by species

Animal species	Number of uses	Percentage
Mice	1,803	89.30%
Zebrafish	216	10.70%
Total uses	**2,019**	**100.00%**

Table 21 shows the uses of animals for the maintenance of established genetically altered lines by severity. 98% of animals used for the maintenance of established genetically altered lines in 2021 were reported as having experienced mild severity.

Table 21: Uses of animals for the maintenance of established genetically altered animal lines by severity

Severity	Number of uses	Percentage
Non-recovery	0	0.00%
Mild [up to and including]	1,979	98.02%
Moderate	30	1.49%
Severe	10	0.50%
Total uses	**2,019**	**100.00%**

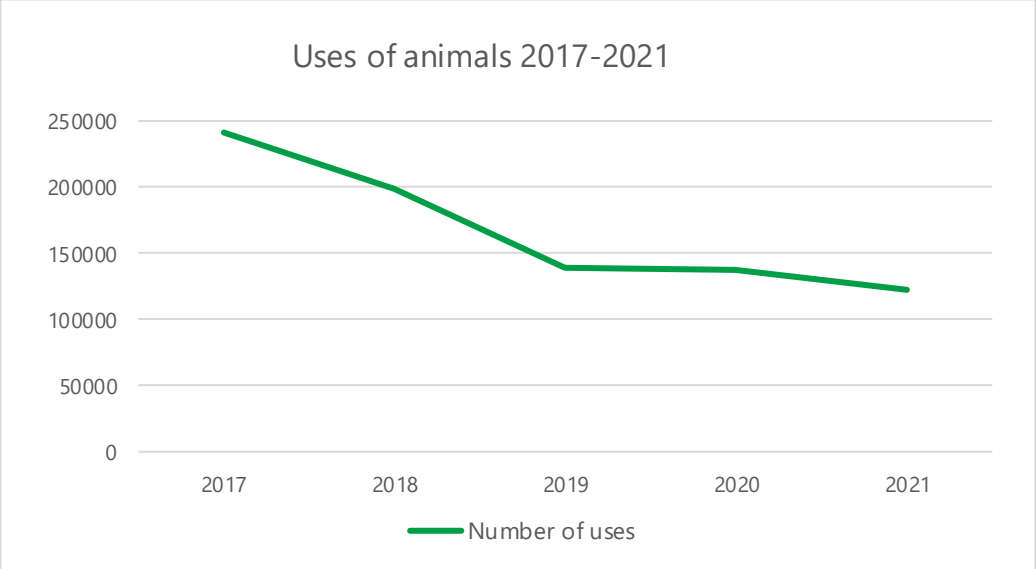
Table 22 shows the uses of animals for the maintenance of established genetically altered lines by genetic status of the animals. 32% of animals used for the maintenance of established genetically altered lines in 2021 were reported as not genetically altered, with 39% reported as genetically altered with a harmful phenotype.

Table 22: Uses of animals for the maintenance of established genetically altered animal lines by genetic status

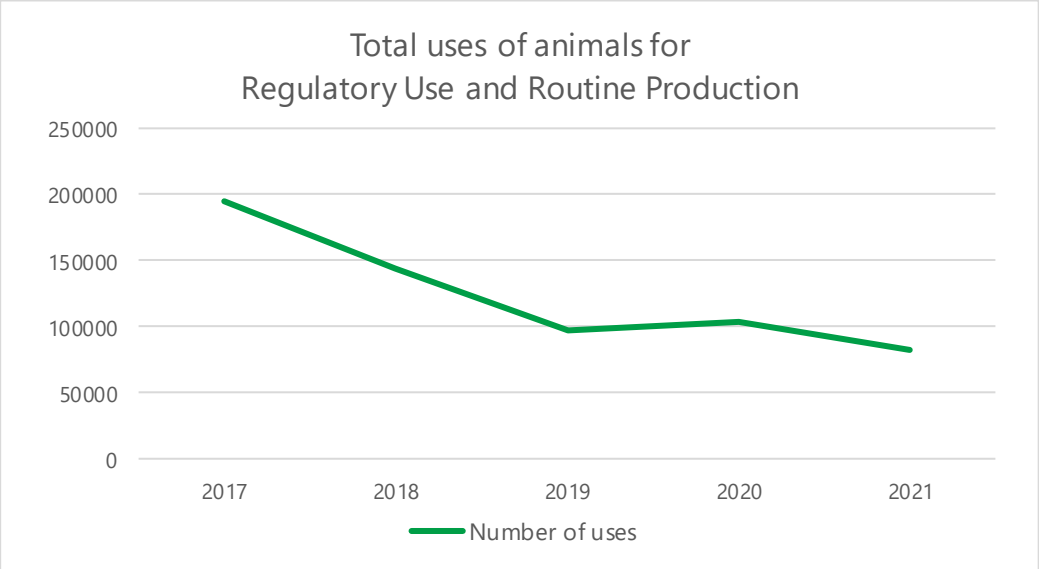
Genetic status	Number of uses	Percentage
Not genetically altered	641	31.75%
Genetically altered without a harmful phenotype	593	29.37%
Genetically altered with a harmful phenotype	785	38.88%
Total uses	**2,019**	**100.00%**

5 TRENDS

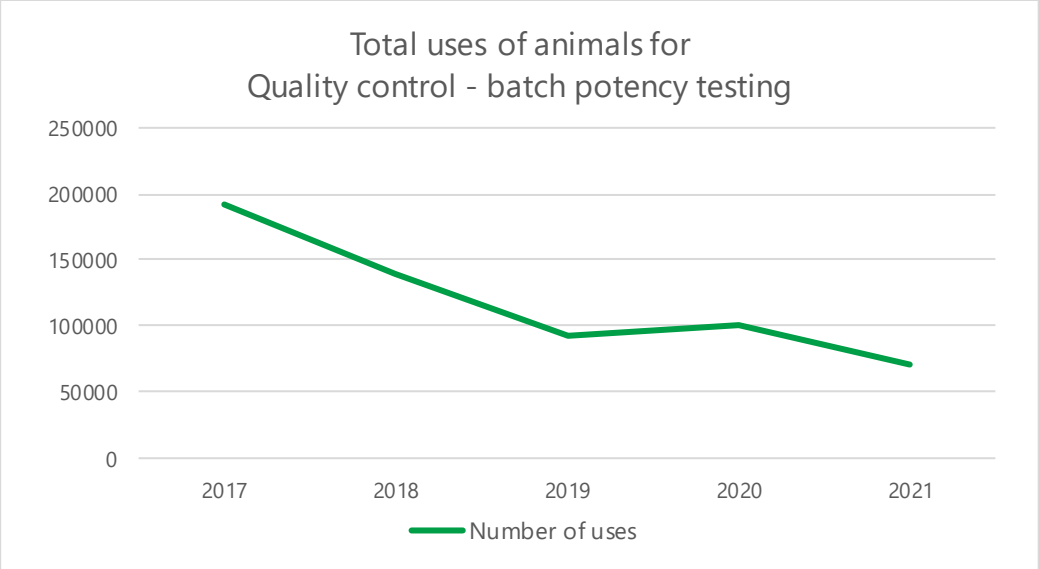
a) In 2021, there were 122,383 uses of animals for research, testing, routine production, and education and training purposes. This represents an 11% decrease from the number of uses reported for 2020 (137,988 uses in 2020). This decrease was mirrored by an 11% reduction in the numbers of animals used for first time in 2021 versus 2020 (121,558 in 2021 versus 137,318 animals in 2020). The total number of uses of animals in Ireland has decreased over the last five years, as illustrated by the graph below.



b) There was a 20% decrease in the number of animals used for 'Regulatory Use and Routine production' in 2021 (82,325 animals) versus 2020 (102,861 animals). There has been a significant reduction in the number of uses of animals for the purpose of 'Regulatory Use and Routine production' over the last five years as illustrated by the graph below.



c) This reduction in the overall number of animals used for regulatory testing purposes results from a significant decrease in the number of animals used for the secondary purpose 'Quality Control – batch potency testing'. 29,588 fewer animals were used for batch potency testing in 2021 (70,896 animals) than in 2020 (100,484 animals), representing a 29% decrease. By way of more long-term comparison, there has been a 63% decrease in the number of animals used for batch potency testing in 2021 (70,896 animals) versus 2017 (192,015 animals), as illustrated by the graph below.



d) There was a notable (eight-fold) increase in the number of animals used for the secondary purpose 'Quality Control – batch safety testing' from 2020 (1304 animals) to 2021 (10674 animals). This is due to the roll-out of COVID-19 vaccines to the marketplace, and the requirement for safety testing using animals as a condition of authorisation for these vaccines.

e) There was a 30% reduction in the number of rabbits used for pyrogenicity testing (657 in 2021 versus 943 in 2020). This reduction is the result of ongoing efforts to replace the use of the pyrogen test with non-animal alternatives. Alternative testing methods to determine the pyrogenicity of medicinal products are recognised under the legislation of the Union. However, these methods are not suitable for certain classes of medicinal products, for example some blood/protein-based medicines. Therefore, in order to meet the necessary regulatory safety requirements for release of certain medicinal products onto the market there is currently no alternative to the use of the rabbit pyrogen test.

f) The number of animals reported as being used for the purpose of 'Basic research' increased by 29% from 2020 (10,455 uses) to 2021 (13,445 uses). This is most likely due to research projects that had been paused or delayed due to the pandemic recommencing in 2021, as public health restrictions lifted.

g) There was a 118% increase in the number of animals (cattle, sheep, and fish) used for the purpose 'Protection of the natural environment in the interests of the health or welfare of human beings or animals' (6,314 uses in 2021 versus 2891 uses in 2020). This increase was driven by two factors;

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

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

h) There was a 20% decrease in the overall number of animals used for the purpose 'Regulatory Use and Routine production'. This was due primarily to a 28% decrease in the number of mice used (67,689 in 2021 versus 93,512 in 2020). However, there were increases noted in the uses of other species for regulatory testing purposes. There was a 38% increase in the numbers of rats used, with 17,050 rats used in 2021 versus 12,330 in 2020. This was due to an increase in the number of rats used for Regulatory (batch potency) testing of a human medicinal (biological) product. There was also a significant increase in the number of guinea pigs used, with 774 used in 2021 versus 228 in 2020. This was a result of increased levels of Regulatory (batch potency) testing of certain vaccines during 2021. Reported uses of ferrets almost doubled from 186 in 2020 to 358 in 2021 as a result of increased levels of regulatory testing of influenza vaccines in 2021.

i) No uses of zebrafish in procedures for research and testing purposes were reported in 2021. All 607 zebrafish used in 2021 were used for the creation and maintenance of genetically altered lines to generate zebrafish embryos for the purpose of 'Translational and applied research – human sensory organ disorders (skin, eyes and ears)'.

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

k) The distribution of animal numbers across the four severity categories in 2021 mirrors that of 2020, with no significant changes noted.

To Note: In addition to routine year-on-year variation in the number and nature of projects authorised, annual trend data can be impacted by a range of other factors which can lead to variation in the overall number of animals used. Decreases, for example, may arise as a result of the ongoing application of the 3Rs principles while certain extraneous developments may result in increases in the levels of animal use. Among the factors that should be considered when assessing annual trend data include:

- The relocation of existing regulatory testing either to or from Ireland.
- The regulatory requirement for the use of animal tests to determine the safety and effectiveness of new human medicines and vaccines developed in response to emerging and significant public health developments.
- The adoption, following regulatory approval, of alternative testing methods including *in vitro* tests.

6 CONCLUSION

In the year 2021, there were 122,383 reported uses of animals for research, testing, routine production, and education and training purposes in Ireland. In addition, 451 animals were reported as being used for the creation of new genetically altered animal lines, and 2,019 animals were reported as being used for the maintenance of genetically altered animal lines.

The distribution of animal numbers across the four severity categories in 2021 mirrors that of 2020, and as with previous years, the most commonly reported actual severity remains at mild.

The HPRA's focus will continue to be on promoting the replacement of tests using animals with suitable non-animal alternative tests, ensuring that the principle of Reduction is applied appropriately when it is necessary to use animals, and refining the husbandry, care and use of animals in procedures. In addition, the HPRA will ensure that animals are used only when there is no equivalent alternative (non-animal) technique available, and the harm-benefit analysis of the proposed use is favourable.

7 APPENDIX: DEFINITIONS

Procedures

The Directive defines a procedure as: "any use of an animal for scientific or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice". This includes the creation and maintenance of any genetically altered animal lines that may result in pain or distress as per the above definition. Each procedure may consist of several stages or techniques for a single scientific purpose, which is then counted as *one* procedure and reported in the year it was completed.

Reuse

Reuse means that following a full recovery from a completed procedure and having been certified by a veterinarian as being returned to full health, that animal can then be enrolled on another project.

Genetically altered animals

Genetically altered animals are those that have been genetically modified, for example, by the introduction (into an animal) of genetic material from another animal, or by 'knocking out' or disrupting an existing gene. Statistics are only collected on genetically altered animals that have an impairment to their well-being from the genetic alteration (a harmful phenotype), or when a new genetic line of animals is being created and the effect on the animals is not yet known.

Actual severity

At the end of the use of an animal on a procedure, the impact of the procedure must be determined and reported as 'actual severity'. This means that the *highest severity* that an

animal may have experienced throughout the course of their time on procedure (rather than the severity at the end or the average severity throughout) must be recorded. Therefore, it is based on the real impact of the procedure, rather than any predicted impact. The legislation defines four categories of actual severity, in order of least to most harmful, as: non-recovery, mild, moderate, and severe.

Non-recovery: This means the entire procedure is carried out under general anaesthesia and, at the end, the animal is humanely euthanised rather than being allowed to wake up.

Mild: Any pain or suffering experienced by the animal is only slight, minor, or temporary and so the animal recovers in a short period of time. This would include an injection, a short period of social isolation, or non-invasive imaging under sedation or anaesthesia (e.g. MRI scanning).

Moderate: Any suffering experienced by the animal is short-term moderate pain, suffering, or distress; long-lasting mild pain, suffering, or distress; or involves a moderate impairment to their well-being. This would include surgery performed under general anaesthesia, repeated injections or blood tests, or the induction of tumours that cause moderate impairment to well-being.

Severe: Severe procedures indicate a major departure from the animal's usual state of health or well-being, and cause long-lasting moderate pain, suffering or distress, or short-term severe pain. This might include toxicity testing under legislation where fatalities may occur, surgical procedures that cause severe post-operative pain, or the breeding of animals with serious genetic disorders.

It should also be noted that procedures that involve severe pain, suffering, or distress that are *long-lasting* are prohibited under the legislation.