HPRA
An tÚdarás Rialála Táirgí Sláinte
Health Products Regulatory Authority

Annual statistical report for animals used in Ireland under scientific animal protection legislation - 2016

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1 INTRODUCTION

The Health Products Regulatory Authority (HPRA) is the state agency with responsibility for regulating human and veterinary medicines, medical devices and other health products. 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, and thus has been publishing statistical data on animals used from 2013 onwards.

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 strongly justified and following independent assessment. The Directive firmly anchors in EU Legislation the 3Rs, i.e. Replacement, Reduction and Refinement:

- **Replacement** means that 'alternative' methods are to be used where possible instead of live animals. Examples of alternative methods would include *in vitro* tests such as the use of cell lines, computer simulation and modelling, video material, or the use of invertebrates such as fruit flies or worms.

- **Reduction** means that it must be ensured that the *appropriate* number of animals is used for *each* project. This allows scientists to obtain statistically robust data without using more animals than are necessary.

- **Refinement** means that the animals used are provided with the best possible care and that suffering is reduced to an absolute minimum. Refinement techniques would include, for example, careful handling by trained individuals, the provision of high standards of housing and husbandry to include enrichment materials (e.g. toys and nesting material) and the appropriate use of anaesthesia and pain relief during procedures.

Although complete replacement of animal studies is the ultimate goal of the Directive, this is not currently possible. 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 in order to safeguard human, animal and environmental health. However, the Directive is a significant tool to protect those animals that are still required.

[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, including unannounced inspections. In 2016, the HPRA performed 33 inspections, of which 50% were unannounced. The HPRA was satisfied with overall levels of compliance nationally.
2. **Projects:** Scientific procedures involving animals can only take place following a detailed submission of the planned study 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 based on the 3Rs and requires scientific justification for the research techniques being applied. The likely impact on the animals must be minimised as far as possible by applying refinements and any harms experienced by the animals must be outweighed by the expected benefits of the work. The HPRA checks whether alternative (non-animal) methods are available or appropriate, as alternatives to the use of live animals must be used where possible. In fulfilment of the HPRA's mandate to promote the 3Rs, in 2016 the HPRA continued its efforts to enhance awareness of non-animal alternatives as well as refinements in the conduct of scientific studies in animals though 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 2016 in accordance with Article 54(2) of the Directive. This is the fourth report to be prepared by the HPRA since it became the competent authority for the protection of animals used for scientific purposes. The Department of Health published all reports in this area prior to 2013 and any project which was originally authorised by the Department of Health continues in force until the expiry of the authorisation in question. This may take up to five years (i.e. until the end of December 2017).

As in previous years, the data provided are based on self-declarations by the establishments concerned. The methodology and legal basis for the requirements for data collection were substantially changed with the introduction of the Directive in 2013. For example:

- Previous data (i.e. prior to 2013) reported only on the first use of each animal, whereas this report includes any subsequent uses of the same animals (reuse).
- Each use of an animal must now be assigned to a specific project purpose outlined by the legislation, e.g. basic research, translational research, regulatory use etc.

- The breeding of genetically altered animal lines was not required to be included in previous reporting years and this is now a requirement of the legislation.
- The actual severity experienced by the animals must now be reported under four categories: non-recovery, mild, moderate or severe.

This format meets the requirements for a European database which has been developed by the European Commission. The data for 2016 has been presented in a slightly different manner to the previous reports published by the HPRA, following clarification as to the precise formatting requirements.

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

2 SUMMARY

a) In 2016, there were a total of 226,934 uses of animals for procedures, with reuse representing 1% of this number. Mice were the most commonly used species at 85% of the total animal use.

b) Of the total number of uses of animals in procedures (226,934), some 170,976 (75%) 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).

c) Of the total number of uses of animals in procedures (226,934), 10,369 involved genetically altered animals, which represents 5% of all animal use.

3 RESULTS

3.1 Species and numbers of uses of animals in procedures

Table 1 shows the number of uses of animals in procedures. It shows both the first, and all the subsequent uses of the animals that were completed in the year 2016. A single use of an animal extends from the time when the first technique is applied to the animal until the completion of data collection, or when the animal is removed from the project. It should be noted that this does not represent the total number of animals used because some animals are reused (see section 3.2).

Mice (85%) were by far the most commonly used species. The next most common species used were fish and rats. The category 'other fish' (5%) primarily represents wild fish being studied for conservation projects. For example, European eels are a critically endangered species and Irish salmon stocks are critically low, so monitoring projects are required to improve the survival of these species. Likewise, the category 'other birds' (<1%) represents wild bird species being studied in monitoring and conservation projects. Dogs and cats were used exclusively in studies for the development of veterinary medicines, which is expected to be of benefit to those species. It should be noted that the following species have been excluded from this table as they were not used in Ireland in 2016: Mongolian gerbils, hamsters, other rodents, other carnivores, reptiles, rana, other amphibians, cephalopods and non-human primates.

Table 1: Numbers of uses of animals by species

Animal species	Number of uses	Percentage
Mice	192121	85%
Rats	9892	4%
Guinea pigs	964	<1%
Rabbits	1228	<1%
Cats	271	<1%
Dogs	356	<1%
Ferrets	404	<1%
Horses, donkeys & cross-breeds	204	<1%
Pigs	1209	<1%
Goats	30	<1%
Sheep	1323	<1%
Cattle	6044	3%
Domestic fowl	196	<1%
Other birds	674	<1%

Animal species	Number of uses	Percentage
Xenopus	60	<1%
Zebrafish	1439	<1%
Other fish	10519	5%
Total uses	**226934**	**100%**

3.2 Reuse of animals

Table 2 shows the proportion of reuse (see Appendix for definition), which represents 1% of animal use. 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). 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 2035 represents *uses of animals,* not actual numbers of animals reused). Cattle are the species that are reused most frequently. In Ireland, cattle are used only for agricultural research studies (for the benefit of the species or the agricultural industry). The overwhelming majority of projects of this nature are of overall 'mild' severity, with only a tiny minority reaching 'moderate' severity. Since the severity cattle experience on these projects is limited, and they return to full general health between projects, it is acceptable that they are reused in further projects.

Table 2: Reuse

Reuse	Number of uses	Percentage
No	224899	99%
Yes	2035	1%
Total uses	**226934**	**100%**

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 (93%) 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 (6%) include wild animals and farm animals. Animals born in the rest of Europe and the rest of the world includes animals that have been obtained from breeding establishments outside the EU (e.g. specific strains of mice not available in the EU) as well as wild animals that have travelled into Ireland from other regions (e.g. migratory birds).

Table 3: Origin of animals

Place of Birth	Number of uses	Percentage
Animals born in the EU at a registered breeder	209791	93%
Animals born in the EU not at a registered breeder	14166	6%
Animals born in rest of Europe	455	<1%
Animals born in rest of world	487	<1%
Total uses	**224899**	**100%**

3.4 Project purposes

Table 4 shows the primary purposes for which animals were used. The most common primary purpose at 75% 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 following primary purposes were excluded from this table as no uses were reported under these purposes in 2016: 'Preservation of species' and 'Forensic enquiries'.

Table 4: Primary purpose for which animals are used

Primary purpose	Number of uses	Percentage
Basic research	28340	13%
Translational and applied research	26230	12%
Regulatory use and routine production	170976	75%
Protection of the natural environment in the interests of the health or welfare of human beings or animals	235	<1%
Higher education or training for the acquisition, maintenance or improvement of vocational skills	250	<1%
Maintenance of colonies of established genetically altered animals, not used in other procedures	903	<1%
Total uses	**226934**	**100%**

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. The most common sub-field of 'Basic research' at 42% was research into ethology, animal behaviour and animal biology. In Ireland, this is mainly accounted for by agricultural research (e.g. nutrition and reproduction studies in farm animals) or research into the behaviour of wild animals (e.g. fish or bird tracking studies). The next most common purpose was research into the immune system (24%). Immune system research involves studies that look at particular cells in the immune system, such as the cells involved in autoimmune diseases e.g. diabetes, multiple sclerosis and asthma, and these types of studies most commonly use mice.

Table 5: Uses of animals for basic research

Basic Research	Number of uses	Percentage
Oncology	213	<1%
Cardiovascular, blood and lymphatic system	803	3%
Nervous system	3876	14%
Respiratory system	1081	4%
Gastrointestinal system including liver	2388	8%
Musculoskeletal system	68	<1%
Immune system	6667	24%
Urogenital/reproductive system	4	<1%
Sensory organs (skin, eyes and ears)	553	2%
Endocrine system/metabolism	101	<1%
Multisystemic	717	3%
Ethology / animal behaviour /animal biology	11868	42%
Other basic research	1	<1%
Total uses	**28340**	**100%**

Table 6 shows 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 welfare. The most common purpose at 36% was research into human nervous and mental disorders, which includes research into treatments for diseases such as epilepsy, autism, and depression, using mice and rats. The next most common purpose, at 29%, was research into animal diseases and disorders. In Ireland this is mostly accounted for by studies investigating diseases of farm animals and fish.

Table 6: Uses of animals for translational and applied research

Translational and applied research	Number of uses	Percentage
Human cancer	1566	6%
Human infectious disorders	947	4%
Human cardiovascular disorders	2086	8%
Human nervous and mental disorders	9308	36%
Human respiratory disorders	80	<1%
Human gastrointestinal disorders including liver	369	1%
Human musculoskeletal disorders	538	2%
Human immune disorders	1130	4%
Human sensory organ disorders (skin, eyes and ears)	1553	6%
Human endocrine/metabolism disorders	584	2%
Animal diseases and disorders	7691	29%
Animal welfare	360	1%
Diagnosis of diseases	18	<1%
Total uses	**26230**	**100%**

Table 7 shows the breakdown of animal uses for 'Regulatory use and routine production'. Regulatory testing refers to procedures carried out with a view to satisfying legal requirements for producing, placing and maintaining products/substances on the market. The majority of reported uses (99%) can be attributed to quality control testing.

Table 7: Uses of animals for regulatory testing

Regulatory use	Number of uses	Percentage
Quality control (including batch safety and potency testing)	169714	99%
Toxicity and other safety testing including pharmacology	1262	<1%
Routine production	0*	0%
Total uses	**170976**	**100%**

*No uses were reported for 'Routine production', therefore no 'Use of animals for regulated production by product type' table is provided in this report.

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 (99%). Batch potency testing is mainly carried out for biological products. This testing is performed to ensure that each manufactured batch of

product is of a consistent strength, to ensure that it will be both safe for patients, and effective. In relation to pyrogenicity testing, although non-animal alternatives have been developed to replace this type of testing for certain medicines, there are still some limited circumstances in which it is not possible to use a non-animal alternative, and therefore it is necessary to continue to employ animal-based tests.

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

Quality control	Number of uses	Percentage
Batch safety testing	1630	<1%
Pyrogenicity testing	506	<1%
Batch potency testing	167549	99%
Other quality controls	29	<1%
Total uses	**169714**	**100%**

Table 9 shows a further breakdown of animal use for 'Toxicity and other safety testing'. The majority (94%) of these tests were for ecotoxicity testing.

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

Toxicity and other safety testing	Number of uses	Percentage
Acute and sub-acute testing	0†	0%
Repeated dose toxicity	0‡	0%
Kinetics	12	<1%
Pharmacodynamics	30	2%
Ecotoxicity	1180	94%
Target animal safety	40	3%
Total uses	**1262**	**100%**

†No uses were reported for 'Acute and sub-acute testing', therefore no additional 'Acute and sub-acute toxicity testing methods' table provided in this report.
‡No uses were reported for 'Repeated dose toxicity', therefore no additional 'Repeated dose toxicity' table provided in this report.

Table 10 shows that animals used for ecotoxicity testing were all reported as being used in acute toxicity tests (the other categories were excluded from this table as no uses were reported). All of the ecotoxicity tests were conducted on fish.

Table 10: Uses of animals for regulatory testing - ecotoxicity

Ecotoxicity	Number of uses	Percentage
Acute toxicity	1180	100%
Total uses	**1180**	**100%**

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. The vast majority (98%) were performed on medicinal products manufactured for use in humans. The category 'other legislation' is accounted for by studies performed under environmental, pollution control and national animal disease control legislation. Table 12 shows the geographical origin of the legal requirement. The majority of tests (82%) were performed to satisfy EU legislative requirements.

Table 11: Regulatory testing by type of legislation

Testing by Legislation	Number of uses	Percentage
Legislation on medicinal products for human use	167227	98%
Legislation on veterinary medicinal products	2540	2%
Other legislation	1209	<1%
Total uses	**170976**	**100%**

Table 12: Origin of legislative requirement

Legislative Requirement	Number of uses	Percentage
Legislation satisfying EU requirements	139897	82%
Legislation satisfying national requirements only [within EU]	29	<1%
Legislation satisfying non-EU requirements only	31050	18%
Total uses	**170976**	**100%**

3.6 Use by genetic status

Table 13 shows the number of uses of animals with 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 were not genetically altered, with less than 1% of all uses involving animals with harmful phenotypes. (Please refer to Appendix for definitions).

Table 13: Genetic status

Genetic Status	Number of uses	Percentage
Not genetically altered	216565	95%
Genetically altered without a harmful phenotype	8360	4%
Genetically altered with a harmful phenotype	2009	<1%
Total uses	**226934**	**100%**

3.7 Use in creation of a new genetic line

Table 14 shows the numbers of animals used in the creation of a new genetically altered line. This represents less than 1% of overall animal use.

Table 14: Creation of a new genetically altered line

Creation of new genetic line	Number of uses	Percentage
No	225555	99%
Yes	1379	<1%
Total uses	**226934**	**100%**

3.8 Actual severity of uses

Table 15 shows the reported actual severity experienced by the animals during their uses in procedures. Overall, 1% of uses were classified as non-recovery, 44% were classified as mild, 22% were moderate and 29% were severe. (Please refer to Appendix for definitions). Of the animals that were involved in severe procedures in 2016, 99% were mice. It should be noted that the severe classification was not exceeded, nor did the HPRA grant any exemptions for the severe classification to be exceeded.

Table 15: Classification of actual severity

Severity	Number of uses	Percentage
Non-recovery	2357	1%
Mild [up to and including]	99442	44%
Moderate	58832	26%
Severe	66303	29%
Total uses	**226934**	**100%**

4 TRENDS

a) In 2016, there were a total of 226,934 uses of animals in procedures, which represents a 1% decrease on the number of uses reported for 2015 (228,975).

b) As in previous years, in 2016 mice were still the most commonly used species representing 85% of overall animal use. Significant changes in species from 2015 to 2016 include the drop in the number of uses of cattle by 46% and the increased use of 'other fish' (e.g. salmon, eels and trout) by 125%. The decrease in cattle use relates to the completion of a particularly large country-wide cattle study in 2015. The increase in the use of 'other fish' relates to the increased tracking of eel and salmon stocks in Ireland (using tagging methods) due to concerns regarding their conservation status.

c) Of the 226,934 procedures completed in 2016, 2,035 involved reuse of animals. This represents a 21% decrease on reuse reported in 2015 (2,582 uses). However, it is not possible from the data to determine the total numbers of animals reused as animals may be reused more than once.

d) The project purpose 'Regulatory use and routine production' accounted for most animal use in 2016 (75%). Although non-animal alternative tests have replaced the need to use animals for regulatory testing for a number of human and veterinary medicinal products, these non-animal alternatives are not available/approved for all medicinal products which require regulatory testing. Therefore there remains in some instances a requirement to perform regulatory testing of medicinal products using animals.

e) 'Basic research' has reduced by 33% from the 2015 figures and 'Translational and applied research' has increased by 14%. These changes are unlikely to represent a true shift in the type of research, but rather may represent more consistent reporting by users as they are becoming more acquainted with reporting requirements.

f) The number of uses of animals reported for 'Maintenance of colonies of established genetically altered animals, not used in other procedures' has reduced by 80% from 2015 to 2016. Whilst the reason behind the significant drop in animal numbers is unclear, it may be potentially due to more consistent reporting by users as they are becoming more acquainted with reporting requirements.

g) In terms of actual severity of procedures, the majority of procedures are still reported as mild (44% in 2016, 50% in 2015). The proportion of moderate procedures has increased from 22% (2015) to 26% (2016) of overall use, and the proportion of severe procedures has increased from 27% (2015) to 29% (2016). Non-recovery procedures have dropped from 2% to 1% of overall animal use. Clear trends in relation to severity

may take a few more years to emerge as users are still becoming acquainted with the new reporting requirements.

5 CONCLUSION

In the year 2016, there were 226,934 reported uses of animals in procedures in Ireland. This is a 1% decrease on the number of uses reported for 2015, and follows a 1% increase between 2014 and 2015. Although this is still a relatively new reporting system, the number of uses appears to be quite consistent over the last 3 years. As with previous years, the majority of animals are still being used for regulatory purposes, and the most commonly reported actual severity is mild.

The HPRA's focus will continue to be the reduction of severe suffering, for example, with the application of earlier humane endpoints, as well as ensuring that the 3Rs are applied to all authorised projects. In addition, the HPRA will ensure that procedures are only performed where there is no alternative (non-animal) technique available and where the expected benefits outweigh any possible harms.

6 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 having fully recovered from a completed procedure, and having been certified by a veterinarian as having 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 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, or 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 and 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 and 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.