Animal Testing Annual Report 2022

Introduction

There are different ways of addressing research questions in biology, sustainable food production and *life sciences* (WUR's area of focus). For example, computer models can be used to make calculations and forecasts. Laboratory tests are suitable for modelling processes or investigating sub-processes in the human or animal body. These methods are developing rapidly and are leading to increasingly better results. But they are not always sufficient. There are times when the search for answers requires animal testing. In some cases, an animal test is even required by law. Animal tests are also used for educational purposes.

Wageningen University & Research (WUR) carries out research into agriculture, nutrition, nature and the environment. The focus of this research may be humans or animals. In either case, animal testing may be used as part of the research. The test animal may be used to represent a human, or the animal itself. In this case, the test animal is the target animal. Research into farm animal health and welfare or the protection of animals in the wild are examples of this. At WUR, the test animal is very often also the target animal. Animal testing is still necessary or even mandatory in certain domains (e.g. vaccine development and monitoring) for scientific and/or public interest reasons.

WUR is persistent in its efforts to replace, reduce and refine animal testing. We also advocate at the European level for alternative methods to be accepted. We are signatories to the Code for Transparency in Animal Testing established in 2008 by the former Association of Universities in the Netherlands (VSNU), now known as Universities of the Netherlands. This means we are committed to transparency regarding the animal tests we conduct. Finally, WUR's Animal Sciences Group has invested in the four-year Next Level Animal Sciences research programme. Any innovations that emerge out of this will help reduce and replace animal testing. More details on this are provided in the 'Next Level Animal Sciences Research Programme' section on page 8.

Contents

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How do we apply for an animal testing licence?

Only researchers who have the relevant training ('Article 9 status') and are employed by an institution that is licensed to carry out animal tests (a licensee) may apply to conduct such a test. However, even if a researcher considers an animal test to be necessary, there is a long way to go before the test can actually start. To be able to carry out an animal test, the researcher first needs to apply for a project licence.

These applications include a detailed description of the research project and the scientific and social importance of the findings. The researcher must present a case for conducting an animal test rather than using an alternative approach. The applicant is legally obliged to describe why an approach that does not use animal testing is not possible, and why it is not possible to carry out the test with fewer animals or in a way that causes less discomfort for the animals. The aim to replace, reduce and refine animal testing (the 3 Rs) is addressed later in this annual report, in the chapter on Alternatives to animal testing. The licence application is assessed in various steps. These steps include looking at issues such as legal requirements, ethical considerations, the scientific/social value of the animal tests, and technical aspects.

Legislation

Is the animal test acceptable under Dutch law?

The European directive on animal testing was drawn up to protect test animals in Europe. This directive has been incorporated into Dutch law as follows:

- Experiments on Animals Act (*Wod: Wet op de dierproeven*)
  The purpose of this Act is to protect test animals. The Act applies the 'no, unless' principle, which means that animal tests may only be conducted if there are no suitable alternative methods for the research project. The Act was amended in 2014, resulting in regulations that are, in some areas, stricter than the European directive. For example, animals that are killed for research in the Netherlands with no prior handling also fall under the protection of the Act.

- Animal Experiments Order (*Dierproevenbesluit*)
  This describes the conditions that companies, institutions and researchers must meet in order to conduct animal tests. It covers issues such as housing requirements for test animals and training requirements for people who work with test animals.

- Animal Experiments Regulations (*Dierproevenregeling*)
  These regulations go into more detail than the Experiments on Animals Act and the Animal Experiments Order, for example by setting out specific requirements that the licensee, researcher and test animal breeder must meet.

Considerations

Does the research aim outweigh possible animal discomfort?

Animals have an intrinsic value, separate from their usefulness to humans. This principle is articulated in the 2013 Animals Act and the Experiments on Animals Act (Wod). When applying for a licence, a researcher must therefore also provide valid ethical arguments for why the animal test is necessary.

Scientific value

Is the test scientifically justified?

The scientific value of the animal test must be maximised and the number of animals and level of animal discomfort minimised. The assessment of the scientific value must include an assessment of the design of the test. For example, it would include looking at the risk of mistakes being made, and exactly how many test animals are needed. It is important not to use more test animals than are needed, but enough animals must be used to enable reliable research results to be obtained.

Technical aspects

Will the test be conducted in the best possible way?

Do the researchers and other staff members have the required expertise and skills, are the housing and care facilities appropriate, and are suitable procedures used?

The licence application procedure is shown in Figure 1 on page 4.

What is an animal test?

According to the Experiments on Animals Act (Wod), a test is considered an animal test if it uses vertebrates or cephalopods (such as squid) and if the animal's suffering consists at a minimum of the insertion of a needle by a skilled technician, or the animal is killed for the purposes of the research with no prior handling.

Not every test involving animals is therefore an animal test. Nutrition research that investigates an animal's preferences, or the relationship between nutrient uptake, growth and faeces composition under normal housing conditions, is not animal testing as understood under the Wod. If a blood sample is taken, or animals are killed to examine tissues or organs, this would be considered an animal test.

At WUR, all research involving animals is assessed by the Animal Welfare Body (IvD) to check whether or not the planned test is covered by the terms set out in the Wod and is therefore a valid animal test. Even if the research is not considered to be an animal test in accordance with the definition of the Wod, the IvD provides the WUR or the researcher with advice on the best way to conduct the research.

Who is involved in the licence application procedure?

Various bodies and committees are involved in the licence application procedure for animal testing. The most important of these are the Animal Welfare Body (Instantie voor Dierenwelzijn, IvD), the Institutional Animal Care and Use Committee (*Dierexperimenten-commissie*; DEC) and the Central Authority for Scientific Procedures on Animals (Centrale Commissie Dierproeven; CCD).

Animal Welfare Body (IvD)

Every licence holder is required by law to appoint an Animal Welfare Body. This body is responsible for advising on animal welfare and on applying the 3 Rs, registering animal tests and for coordinating the project proposal prior to applying for a licence. The IvD assesses whether the proposed research can be carried out appropriately in the research facility. Attention is also paid to the expertise and skills of the researchers and other staff members, the housing provided for the animals, and the availability of suitable test procedures. Once a project licence has been awarded, the IvD then assesses whether specific animal tests meet the conditions of the licence. In accordance with the Wod, the members of the IvD must meet certain criteria. For example, the IvD must include at least one research scientist and an expert in animal testing – an 'Article 13f3a officer'.

Institutional Animal Care and Use Committee (DEC)

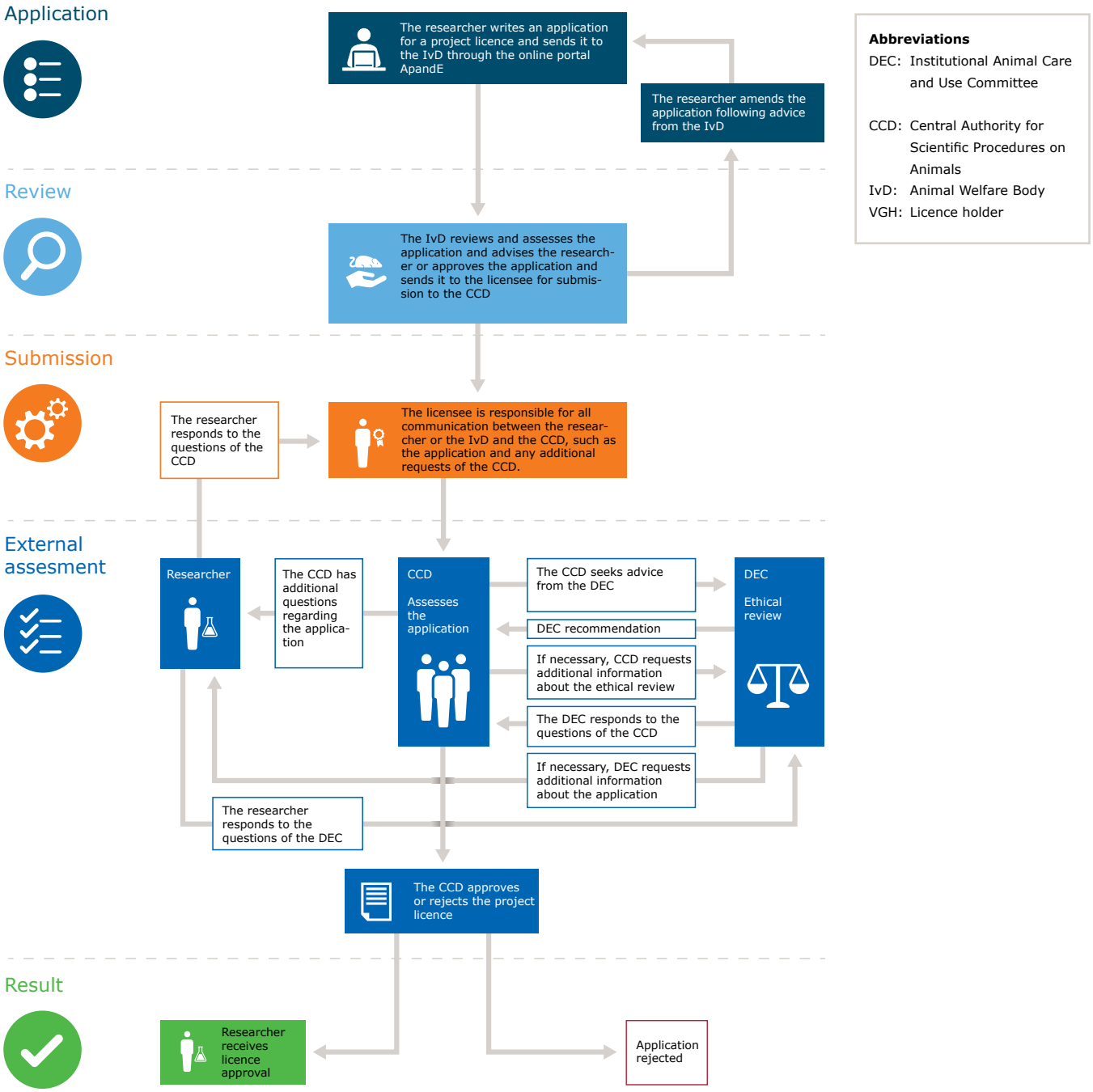
The Institutional Animal Care and Use Committee is an independent committee that evaluates the ethical aspects of applications for a licence to conduct animal tests. The main question that the DEC asks is, 'Does the purpose of the animal test outweigh the discomfort of the animals in question?' To answer this question, the DEC's assessment again includes a consideration of the 3 Rs (more on this in the following chapter):

- Replace: are there alternatives to the animal tests?
- Refining: Can pain or discomfort be prevented (e.g. by modifications to housing)?
- Reduce: Can the number of test animals be reduced? The fewer animals used, the better, but too few animals can mean that the research results are invalid and that a test was carried out for nothing.

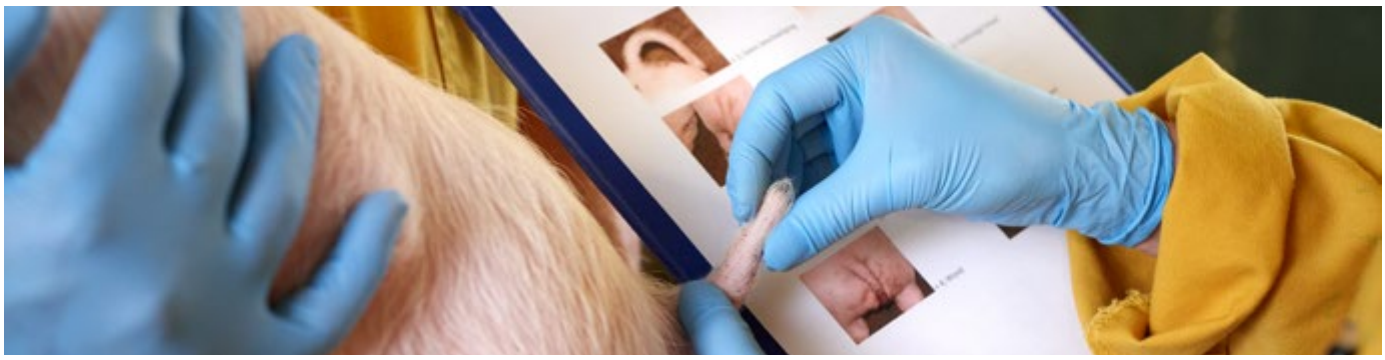
Under the Wod, the DEC must meet certain requirements in terms of expertise and independence. A DEC must be recognised by the Central Authority for Scientific Procedures on Animals (CCD).

Central Authority for Scientific Procedures on Animals (CCD)

The Central Authority for Scientific Procedures on Animals (CCD) is the body that provides licences for animal testing. The CCD is an independent executive body (zelfstandig bestuursorgaan, or ZBO). The CCD always seeks advice from a DEC. Regardless of whether the DEC's view is positive or negative, the CCD will often ask additional questions of the researchers. It then makes its own assessment, with the DEC's recommendation carrying significant weight. In the past the CCD also published the Non-technical Summary (NTS) for the project, which was a brief description of the project, written for a general audience. These summaries are now published on an EU website: ALURES NTS Database.



The Netherlands Food and Consumer Product Safety Authority (NVWA) is the body authorised by the government to take responsibility for monitoring and enforcing regulations in animal testing in the Netherlands. The NVWA performs checks at institutions that carry out animal tests. This includes checking that the necessary licences are held and that the animal tests are performed in accordance with these licences, that the animals are housed and handled in accordance with regulations, and that administrative records are correct. Institutions report annually to the NVWA the numbers of animal tests carried out, and their purpose. The NWVA publishes these figures in an annual report ('Zo doende').



Type of research and use of animal tests

Animal tests are used in a number of different WUR departments and research institutes to address a range of research questions. They are used as part of teaching, scientific research and legal obligations in relation to:

- Human and animal health and well-being
  Test animals may be used as a model for humans as part of research into health and well-being. Examples include research into nutrition and health, lifestyle, health in old age and metabolic disorders. These all concern humans. But the test animal can also serve as a model for the animal itself. In those instances the test animal is known as the target animal (and the research is referred to as 'target animal research'). For example, chickens are used as test animals for research into improving chicken health and well-being. This category also includes research into diseases that can be transmitted from animals to humans, known as zoonoses. In its supplementary guidelines, WUR has stated an aim to be a leading player in target animal research, due to its special expertise in this field. It goes without saying that much of this research does not make use of animal testing.

- Sustainable food production
  Some of Wageningen's research focuses on sustainable food production and reducing its ecological impact. This includes, for example, research into how existing husbandry systems can become more sustainable. New husbandry systems are also being developed that contribute to sustainable food production. Animal husbandry research covers topics such as nutrition, the environment, and animal behaviour and welfare.

- Animal health, food safety and the risks associated with environmental pollution
  A large part of WUR's research into animal health, food safety and the risks associated with environmental pollution is statutory research that is commissioned by the Dutch government. These are the 'statutory research tasks'. Examples include the diagnosis and identification of notifiable animal diseases, safety and efficacy tests, identifying trace contaminants, and toxicology research. Wageningen also carries out research into the toxicity of substances in the environment. This research directly contributes to the provision of safe environments for humans and animals.

- Sustainable management of the environment
  The monitoring of fish stocks contributes to the sustainable management of terrestrial and aquatic environments, such as our oceans and forests. Research

8 WUR guidelines and ambitions for animal testing

All animal tests at Wageningen University & Research comply with the Experiments on Animals Act. In addition, in 2019 WUR formulated its own animal testing policy which consists of the following 8 guidelines and ambitions.

1. WUR only conducts animal tests that are appropriate to WUR's domain and mission
2. WUR continuously invests in the application of the three Rs (Replacement, Reduction, Refinement)
3. WUR sees itself as an organisation with special expertise in the field of animal testing in which the test animal serves as a model for the animal species itself (target animal research)
4. WUR advocates at the European level for alternative methods to be accepted
5. WUR trains its students in the responsible use of test animals
6. WUR is transparent about its use of test animals and engages in dialogue about the responsible use of test animals
7. WUR ensures that training requirements are met as stipulated in the Experiments on Animals Act (Wod) including further and additional training (lifelong learning)
8. When working in international partnerships, WUR's researchers adhere to the Netherlands Code of Conduct for Research Integrity

More information on these guidelines and ambitions is available at wur.nl.

into the reintroduction of threatened species such as the otter, and ecological research into the behaviour of wild animals, such as the great tit, is also included in this category. The use of transmitters or trackers, such as those used to monitor fish stocks, can be considered an animal test in that context. The use of animal tests to develop our knowledge in the ecological domain improves our ability to protect animals and species from human influences. This enables us to ensure that those animals have sufficient access to habitats, and also enables us to maintain or even to improve biodiversity.

- Plant health
  A very small proportion of the research projects that use animal testing concern plant health. These projects include the development of tests to detect plant diseases. Every year, depending on the crop, between 15 and 50% of the global harvest is lost to disease and plague. If we are to improve food security, it is vitally important to prevent plant disease. In the Netherlands alone, millions of plant samples are tested every year using tests developed at WUR. These tests are just as important at the global level. A lot of research is being conducted into the development of alternative tests to identify plant diseases without the use of animal testing.

- Development and validation of alternatives to animal testing
  Test animals are also required for the development and validation of alternatives to animal testing. This is because an existing animal test is often required for comparison purposes (as a 'reference') to demonstrate the reliability of the alternative. The careful validation of animal testing alternatives is important to increase acceptance of the alternative both within WUR and beyond.

Chicken welfare research

Research into the welfare of chickens focuses for example on increasing their resilience. This improves their ability to cope with environmental challenges such as infections, dietary changes or transportation. In turn, this enhances their welfare in production settings.
Research conducted in 2018 included looking at the effect of early-stage rearing conditions for broilers on their behaviour, immunological traits and the composition of the gut microbiome (profile of gut bacteria). Chicks were given optimal conditions until day 14 by hatching them in the barn and giving them a surrogate mother. This surrogate mother consisted of a warm and dark resting place. These chicks were compared with control chicks that were not given such facilities. For this research project, the researchers used an animal model under experimental conditions. This allowed them to scrupulously monitor behaviour in the coop, the composition of the microbiome in different segments of the intestine, immunological characteristics, and the chicks' response to a mild stressor in a behavioural test. For example, they would remove the chicks from the group for two minutes and note how often a chick chirped in that situation. The more a chick chirps, the more stressed it is. This provides insights into underlying mechanisms of resilience and health, as well as possible associations between the microbiome composition, the response to the behaviour tests and the expression of natural behaviour in the coop, such as scratching in the dirt, preening and taking dust baths.

Human health research using mice

Many human diseases are characterised by excessive fat storage in cells. For example, an accumulation of fat and inflammation in the liver (steatohepatitis) is associated with an increase in fat storage in the liver cells. Researchers at WUR want to better understand how cells deal with fat. They want to know why excess fat is harmful to most cells and how it could be prevented. To do this, they look for new factors that influence the storage of fat in cells. They have been able to show that a certain protein, HILPDA, increases the accumulation of fat in cultivated liver cells. However, they do not know whether HILPDA has the same role in the human liver. To find this out, they use mice as an animal model for humans. This is a special type of mouse that is no longer able to produce the HILPDA factor. They examined whether the absence of HILPDA affects the storage of fat in tissues and whether this has consequences for the development of certain diseases, such as steatohepatitis and diabetes. Initial results show that the absence of HILPDA does in fact result in less fat accumulation in the liver. The next step is to examine what effect an absence of HILPDA has on other cells, such as fat cells and white blood cells.

Alternatives to animal testing

WUR is committed to replacing animal testing with other research methods as much as possible. This is not only because WUR recognises the intrinsic value of animals, but also because looking at alternatives can be necessary for the research itself – for example, when researching populations of endangered species. After all, you cannot use animals from the actual population for this, as the research to protect them would itself then harm the population.

These days, a lot of research can be done through alternative methods, for example using tissue culture or computer modelling, and these methods can even be superior and cheaper (replace). Where replacement is not possible and animal testing is necessary or even a legal requirement, we conduct tests using as few animals as possible (reduce). We also constantly work to minimise animal discomfort and to make the lives of test animals as pleasant as possible (refine). The following sections provide more details on the principles of the 3 Rs, and also show how this is embedded in what's known as the 'OMA policy'.

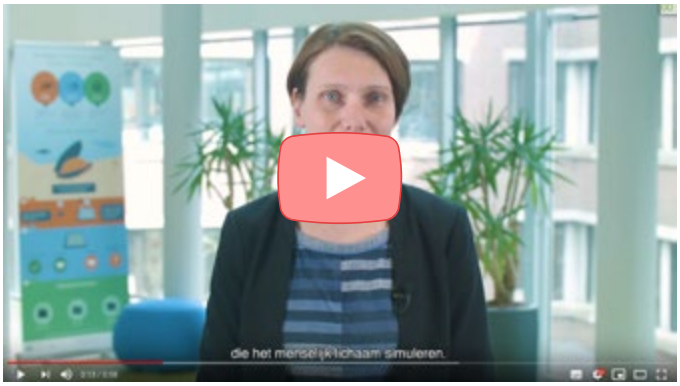
OMA policy and the 3R principle

In late January 2023, the Minister of Agriculture, Nature and Food Quality (LNV) briefed the House of Representatives by letter about progress on the 'transition to animal-free innovation' (TPI) dossier and the animal testing policy. One of the topics addressed in the letter was something known as the 'OMA principle', meaning: focus on the research question ('*Onderzoeksvraag*'), select the best Method, and Analyse.

This principle focuses on selecting the most appropriate method to answer a research question. In practice, it means using tailor-made approaches that combine animal studies, 3R methods, and methods that do not use test animals. The process of choosing the best method is based on the 3R principles. The OMA policy has widespread support among politicians and civil society.

An alternative research model or technique can mean that the use of test animals is delayed until the final, decisive and/or evaluative phase of the research project, or that it is no longer needed at all. In some cases, alternative research methods produce better results and are also cheaper than animal tests.

The video below sets out an example of such a scenario.



Replace

Replacement is when an animal test is fully or partially replaced with computer models or laboratory tests on tissues. In some cases, so much information can be obtained in this way that fewer or no test animals are needed for the actual research project.

Reduce

Reducing is about using as few test animals as possible to obtain a reliable research result. Statistical methods are an important part of this. Improved research methods or test conditions can reduce unintended variants, allowing a reliable result to be obtained using fewer animals.

Examples of replacing animal tests

For toxicological testing and the diagnosis of infectious diseases, animal tests are increasingly being replaced with alternatives, in the form of chemical and biochemical tests. Using organoids – small models of organs made from the relevant organ cells – it is possible to conduct tests on human or animal cell tissue. Organoids cannot replace test animals entirely, but they offer plenty of opportunities for reducing the number of test animals required, especially in preliminary studies.

Alternatives to animal testing are also available for wild animals, for example to investigate the prevalence of a certain species in an area. In the past, researchers had to catch animals, but we can now obtain information such as individual identification, sex and family relationships by analysing the DNA in the faeces.

Refine

There are different ways of refining animal tests. The welfare of test animals can be improved by adapting housing, using remote monitoring (telemetry) to observe the animals, and/or improving laboratory techniques so that less material (e.g. blood) is required. Researchers can also use protocols and procedures that not only address the needs of the animal but also improve the quality of animal-animal and human-animal relationships (e.g. between the test animal and its carer). Another way of improving the welfare of the test animal is to introduce measures that reduce boredom and prevent stress.

Next Level Animal Sciences research programme

This annual report is an opportunity for us to highlight the Next Level Animal Sciences innovation programme. Under this programme – which WUR is investing in from its own resources – animal scientists at Wageningen University & Research are working with partners to develop new research methods and technologies that will play a significant part in reducing, refining and replacing animal tests. The four-year innovation programme (2020-2023) has a budget of €12 million and consists of three lines of research: sensor technology, complex cell systems, and data and models.

Sensor technology

Sensor technology enables continuous and real-time monitoring of animal health and welfare by recording a variety of physiological 'parameters' and behaviour, both in individual animals and groups. Innovative, smart forms of sensor technology are an alternative to invasive measurement methods and animal testing. These include, for example, sensors capable of *real time* monitoring of the energy system and physiological status of animals without taking blood tests or other samples from the animal. Or microcapsules that can be swallowed by a pig and allow *real time* data to be collected from inside their gastrointestinal tract, without causing any discomfort to the animal.

Examples:
Capsules improve the intestinal health of pigs
Artificial intelligence improves welfare of cows

Complex cell systems

Complex cell systems – or organoids – are miniature versions of human and animal tissues and organs. The work being done within NLAS includes developing respiratory organoids from pig and cattle tissue to study respiratory infections and to test therapies. Animal organoid models are also being developed for muscles, neurons and the gut. The purpose of this line of research is to develop innovative and non-invasive research methods to improve the health, welfare and resilience of animals.

Example:
Testing fish feed and vaccines on mini-guts of farmed fish

Data and models

The rapid development of the kind of sensors and camera technology described above is increasingly leading to the creation of larger and more complex datasets, often based on a variety of sources. The challenge is to turn this data into usable and meaningful information about animal behaviour, health and welfare.

As part of this research we are using case studies, courses and network building to develop and disseminate up-to-date knowledge on transmitter data analysis, mechanistic modelling, automatic pattern recognition and complex data analysis.

Example:
Analysis of tracking data and sensor platforms

Accelerating validation

While striving to reduce animal testing as much as possible is a high priority, alternative methods must be at least as reliable as an animal test. Alternatives to animal testing must be validated before they can be used. This can be a lengthy process: for alternatives to be accepted, they must go through organisations at both the European level (ECVAM, the European Centre for the Validation of Alternative Methods) and the global level (OECD, the Organisation for Economic Cooperation and Development).

Reducing discomfort in animal tests through refinement

Refinement is about optimising the welfare of test animals and reducing their discomfort. This concerns not just the animal tests, but the whole process, including transport and the acclimatisation period. Multifunctional test animal housing is available at WUR, in which the housing system is adapted to the specific needs of each type of animal. Everything is focused on maximising each animal's opportunity to express its natural behaviour despite the demands made by the research. For example, litter and other forms of enrichment are added. Pigs will use litter on a daily basis to make a place to rest and sleep. Rodents are provided with shelters and nesting material, and ferrets with hammocks and cloths. Brushes, water bowls and games may also be added. Toys for chickens can include hanging bells and mirrors. Wherever possible, test animals are housed as a group, with extra attention paid to acclimatisation, socialisation and training. Such measures help prevent chronic stress amongst test animals. We are learning more and more about the effects of chronic stress on the immune system and well-being of animals. Preventing chronic stress not only improves animal welfare, but also the quality of the research. The animal carers at WUR are trained to monitor animal welfare.

WUR has already achieved several successes in validating alternatives to animal testing. For example, Wageningen Food Safety Research has developed a chemical-analytical method to detect marine biotoxins in mussels. These biotoxins are algae that can cause diarrhoea, vomiting and even neurological disorders. Testing mussel extracts for the presence of these toxic substances initially involved injecting 300,000 rats a year in the EU and checking whether they died from it: a cruel method. The method developed by WFSR has now been adopted into European legislation.

The validation of an alternative can run in parallel with an animal test as a way of accelerating the process of validation and the use of alternatives.

Figures on animal testing

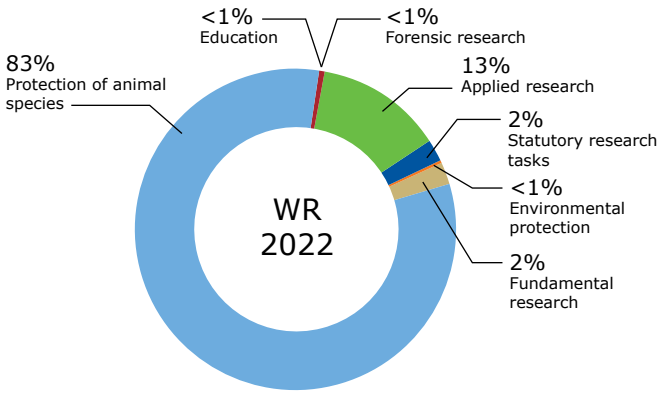
A total of 104,891 animal tests were carried out at WUR in 2022, an increase of 29% compared with 2021. This increase is attributed to the increase in the number of test animals used at Wageningen Research (from 68520 to 94198) for the purpose of protecting animal species such as glass eels. More details are given on this below. All these tests are covered by the Experiments on Animals Act (Wod) and were completed in 2022. Note that the number of animal tests is not the same as the number of test animals used, because more than one test may be carried out on a single animal. The number of test animals used is always less than the number of animal tests conducted.

In 2022, 80% of all animal testing at WUR involved fish. These tests are mainly related to monitoring fish stocks and come under the theme of 'protection of animal species'. In second place are chickens (domestic fowl 12.9%), and mice come third (3.1%). Chickens were mostly used for research into animal welfare. More details on that are also provided below. Not all of the animals used in animal tests are housed in WUR facilities. Research into the conservation or monitoring of populations in the wild can only be done using wild animals. Some animal tests are also carried out on experimental farms, for example on chickens.

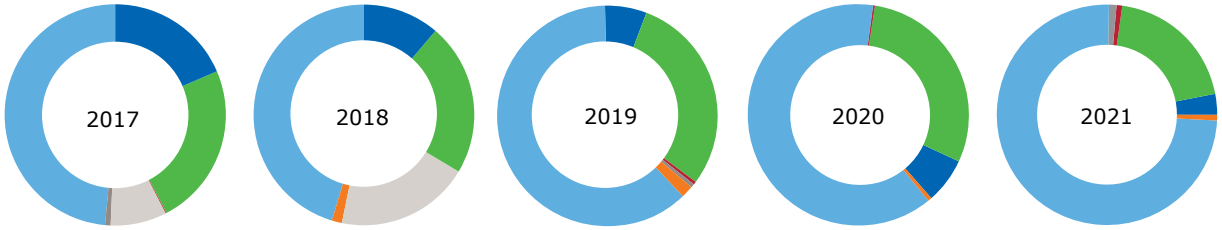
Wageningen Research (WR)

The increase in animal testing at WR is due to different types of research. The biggest increase is in the number of fish used. Most of the animal tests carried out at WR in 2022 were for research into the protection of animal species (83%), primarily the monitoring of fish stocks.

This research is carried out on behalf of authorities such as the Ministry of Agriculture, Nature and Food Quality (LNV), Water Boards and/or provincial governments. These entities want to be able to protect vulnerable fish populations such as glass eels. This research is running from 2019 to 2023. WUR is conducting research to find out which segments of fish populations are being negatively impacted by human activity (as indicated by mortality or delayed development), what the key problems are that affect migration, what problems cause the greatest delays, the effect of interventions to address these problems, how fish respond to the problems, and the factors that determine certain behaviours. This allows us provide evidence-based advice to authorities.
As glass eels are relatively small, no non-invasive transmitter can be used, but they are marked subcutaneously with a needle under anaesthesia. This means the research is considered an animal test. Depending on the location, anywhere from a few hundred to tens of thousands of fish have to be tagged to ensure that a certain amount will be recaptured. This has led to a sharp increase in the number of fish used.



Applied and translational research came in second (13%), after research aimed at the protection of animal species. This was mainly research into animal diseases, such as avian influenza (bird flu), and animal nutrition. Statutory research tasks took third place (1.8%), particularly work on Bioaccumulation (accumulation of hazardous substances in organisms).



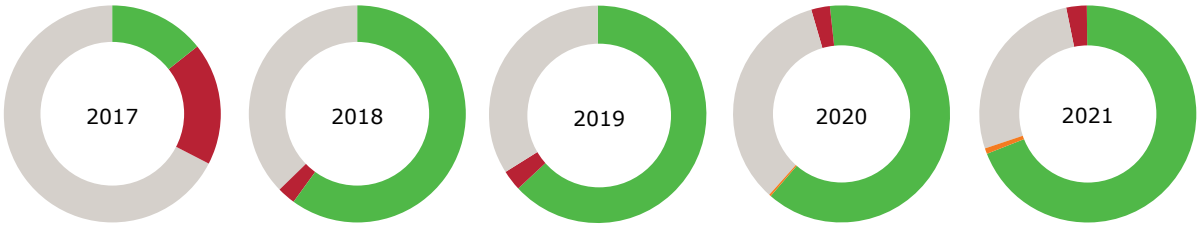
	2017	2018	2019	2020	2021
Environmental protection	0% (0)	1% (695)	1% (479)	<1% (575)	<1% (0)
Protection of animal species	49% (21666)	45% (21044)	63% (40770)	72% (37589)	76% (52308)
Forensic research	<1% (361)	<1% (16)	<1% (4)	<1% (26)	<1% (156)
Fundamental research	8% (3670)	20% (9123)	0% (0)	1% (10)	0% (0)
Education	<1% (58)	<1% (29)	<1% (70)	<1% (67)	<1% (32)
Applied research	24% (10607)	22% (10383)	30% (19144)	23% (12286)	20% (13945)
Statutory research tasks	19% (8283)	11% (5212)	6% (4160)	4% (1936)	3% (2079)

Wageningen University (WU)

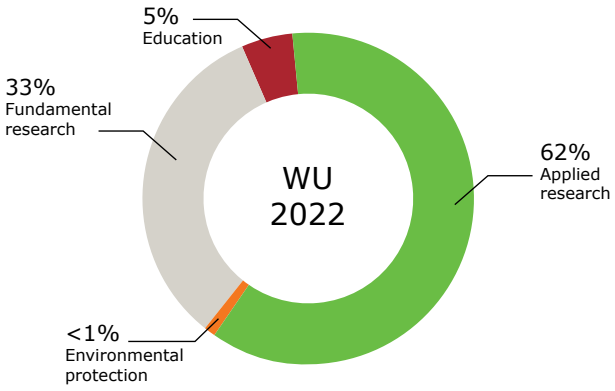
The number of test animals at WU fell from 12948 to 10693. That is a 17% decrease. This decrease applies to a variety of animal species: pigs, rodents and fish.

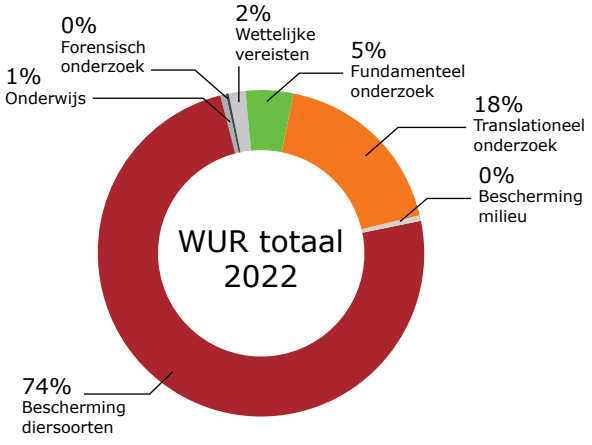
Most of the animal tests carried out at WU in 2022 were for Applied and translational research (62%), in particular research into animal welfare. This relates to the target animal research mentioned earlier in this report. Examples include tests on food additives for chickens, to improve their gut health and overall health.

This was followed by fundamental research (33%), in particular research into the immune system and animal behaviour. Education was third (5%).



	2017	2018	2019	2020	2021
Environmental protection	0% (0)	0% (0)	<1% (46)	<1% (21)	<1% (29)
Fundamental research	67% (4417)	37% (3728)	34% (4458)	34% (2774)	27% (3516)
Education	18% (1195)	3% (273)	3% (383)	2% (149)	3% (415)
Applied research	14% (938)	60% (5999)	63% (8274)	64% (5164)	69% (8988)





Education

Wageningen graduates may be required to conduct animal tests in the course of their careers. WUR therefore believes it has a duty to teach students about responsible animal testing and the ethical aspects associated with the use of test animals. Any students who are opposed to animal tests on ethical grounds or who do not wish to use materials taken from animals during practical sessions may choose to follow a dissection-free variant of the compulsory subjects. This is not however possible for subjects in the specialisation phase of the programme, although students may choose subjects in which they do not have to carry out animal tests. WUR also sometimes uses animal tests to teach students how to handle animals, even if they only come into contact with non-test animals.

Which animals are covered by the Wod?

This law covers animals that are intended to be used for scientific or educational purposes and that will experience discomfort as a result of the test. The law applies to vertebrate animals, including fish and reptile larvae that feed independently, mammalian foetuses from the third trimester of normal development onwards, and cephalopods (such as squid). Some animals held by WUR are not covered by the Wod and are therefore not included in this annual report. This might be because they are kept for breeding, or as field animals, or because the research causes no discomfort – if, for example, they are merely being observed for their behaviour.

Adoption

The 'rehoming' of test animals is permitted under certain conditions. When rehoming these animals, WUR follows the Code of Practice drawn up by the Netherlands National Committee for the protection of animals used for scientific purposes. Cats that are kept at WUR are 'retired' after seven years and put up for adoption. A detailed questionnaire is used to match the right cat to the right owner. The cats that are housed at WUR are mainly used for behaviour and food research. They receive a lot of attention from students and carers and are therefore well-socialised and suitable for adoption.

Overview of test animals used in 2022

Animal species	WU	WR	total
Mice	890	2361	**3251**
Rats	17	2	**19**
Guinea pigs			**0**
Golden hamster		380	**380**
Other rodents[1]	655		**655**
Rabbits			**0**
Dogs		260	**260**
Cats	46		**46**
Ferrets			**0**
Pigs	82	1186	**1268**
Sheep		34	**34**
Cattle	129	552	**681**
Other mammals[2]	129	287	**416**
Domestic fowl	5280	8296	**13576**
Other birds[3]	115	236	**351**
Reptiles[4]			**0**
Zebrafish	1023		**1023**
Other fish[5]	2327	80604	**82931**
	10693	**94198**	**104891**

1 Bank vole, common wood mouse, field mouse, field vole, house mouse (wild caught)
2 Horses, donkeys and cross-breeds, llama, greater white-toothed shrew, common shrew, crowned shrew, Eurasian pygmy shrew
3 domestic duck, goshawk, skylark, buzzard, yellow-browed warbler, goldcrest, starling, great tit, blue tit
4 Salmon, trout, arctic char, grayling, gup, green swordtail, molly, platy, yellowtail kingfish, carp, tilapia, sole, sea bass
5 ≥65 species

	2017		2018		2019		2020		2021	
Animal species	**WU**	**WR**	**WU**	**WR**	**WU**	**WR**	**WU**	**WR**	**WU**	**WR**
Mice	537	6961	1146	4572	640	3082	244	1384	815	2258
Rats	17	0	97	0	69	0	8	30	11	0
Guinea pigs	0	1394	0	485	0	52	0	10	0	152
Golden hamster	0	0	0	0	0	0	0	0	0	1337
Other rodents[6]	0	59	101	70	543	238	21	66	1354	0
Rabbits	0	20	0	29	0	12	0	5	0	0
Dogs	0	17	0	4	0	0	0	10	0	0
Cats	0	0	32	0	16	0	0	32	15	0
Ferrets	0	40	0	0	0	24	0	24	0	0
Other predators[7]	0	263	0	74	0	62	0	0	0	0
Horses, donkeys and crossbreeds	0	6	0	6	0	0	0	0	0	0
Pigs	557	935	1397	1716	753	1952	655	1243	566	2367
Goats	10	36	22	40	0	0	0	0	0	0
Sheep	0	118	0	126	0	119	0	95	0	43
Cattle	223	568	463	696	98	1791	222	2240	80	1788
Other mammals[8]	3909	4	5	6	0	3	0	12	5	30
Domestic fowl	0	6525	3342	5652	4388	12975	2359	6357	5244	6021
Other birds[9]	0	20	0	282	0	164	134	282	0	86
Reptiles[10]	9	277	0	125	0	0	0	360	0	0
Frogs	0	0	0	0	0	0	0	0	0	0
Other amphibians[11]	0	0	0	0	0	41	0	0	0	0
Zebrafish	0	0	843	0	1150	0	602	0	180	0
Other fish[12]	1288	27402	2552	32619	5504	44112	3863	40400	4678	54438
Total	**6550**	**44645**	**10000**	**46502**	**13161**	**64627**	**8108**	**52550**	**12948**	**68520**
Total WUR		**51195**		**56502**		**77788**		**60658**		**81468**

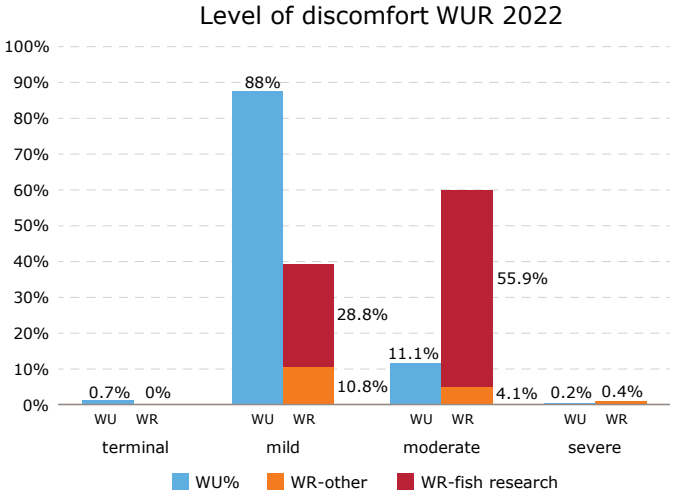
6 Common wood mouse, Yellow-necked mouse, Field vole, Bank vole, Eurasian pygmy shrew, Garden dormouse, Hamster, Hazel dormouse
7 Common seal, Grey seal, Mink
8 Wildebeest, Llama
9 Marsh harrier, Sandwich tern, Goshawk, Pekin duck, Wigeon, White-tailed eagle
10 Slow worm, Common lizard, Turtle
11 Moor frog
12 88 species

Level of discomfort in animal testing at WUR

The table below shows the level of discomfort experienced by test animals during animal testing at WUR in 2022. An expected level of discomfort is estimated as part of the project plan, with a final assessment afterwards. The table shows the actual levels of discomfort. Various factors are taken into account to determine this, such as the pain and fear that a particular procedure causes, or lasting harm to the animal. Animal discomfort is assessed cumulatively. Sometimes different treatments are carried out within a single procedure, with each causing 'mild discomfort'. But because all the treatments throughout the procedure are taken into account, the entire procedure may then fall into the 'moderate discomfort' category, for example.

The tables below show that the percentage of research with moderate discomfort increased at WR, but decreased at WU. The increase in the percentage of moderate discomfort at WR in 2022 compared to previous years is due to the nature of the research into fish stocks conducted at WR. Research into animal diseases, which in some cases involves infecting animals with pathogens to then study the disease, also causes moderate discomfort. For more information, see the box on fish research on page 14.

The increase in the percentage of moderate discomfort indicates a failure to achieve the intended refinement in the research. Moderate discomfort is mainly experienced as part of fish stock monitoring research.
The discomfort experienced by test animals in research conducted by WU has decreased compared to previous years: there is a higher percentage of mild discomfort and a lower percentage of moderate discomfort. This indicates a successful response to the aim of refining the research.



Compared to the national average, WU conducts a relatively large number of animal tests characterised by mild discomfort: 57.8% nationwide (2021 figures) compared to 88% at WU (2022 figures, in 2021 this was 79%).
The national average for moderate discomfort in 2021 was 35.6% while at WU it was 11.1% in 2022 (and 21% in 2021).
About half of the number of test animals used at WU are chickens (domestic fowl). Almost all research on chickens relates to animal welfare research and involves mild discomfort.

Animal tests are assessed as **terminal** if the animal is killed to examine tissues or organs without undergoing any prior treatments. The animal does not therefore experience pain or discomfort during a procedure.

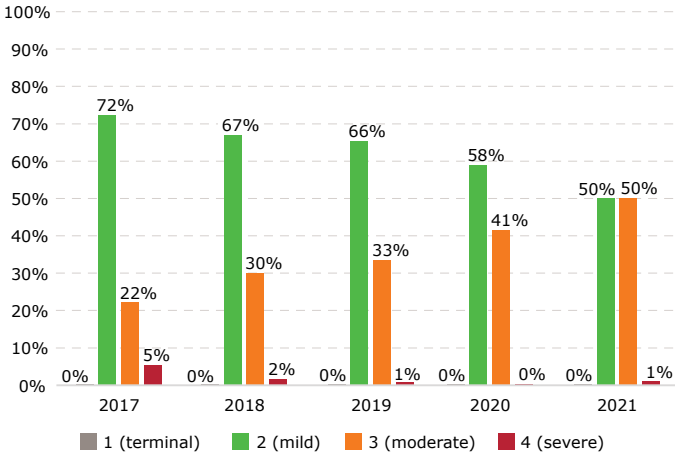
Mild discomfort means that there is a risk of slight pain or discomfort for a short period. These activities and procedures do not therefore significantly affect the animal's well-being. These could include the administration of and waking from a light sedation, taking a blood sample, or administering a substance through a tube. Brief solitary confinement of social animal species such as mice or chickens is also counted as mild discomfort.

With **moderate** discomfort, there is a risk that the animal briefly experiences a moderate level of discomfort, or mild discomfort for a longer time. Examples of procedures that cause moderate discomfort are frequently taking blood samples, surgery with good post-operative pain management, or a number of days (depending on the species) of solitary confinement of social animals.

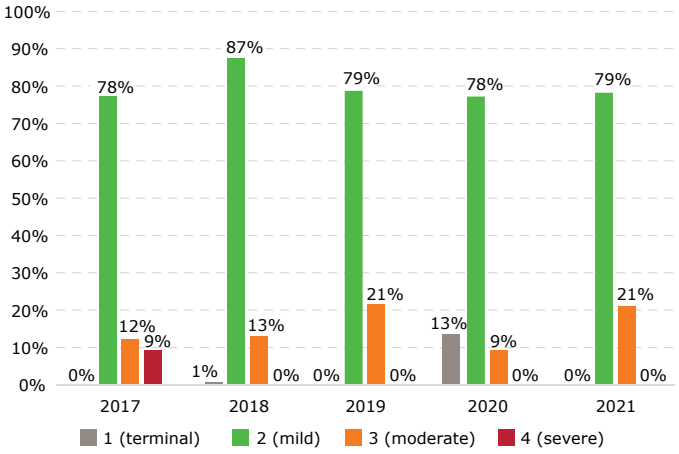
Severe discomfort is the highest category of discomfort. The animal is likely to experience severe discomfort during a test, to the extent that it would seriously harm the animal's well-being. Examples of procedures that cause severe discomfort are exposure to a deadly disease associated with prolonged pain and discomfort, or keeping a social species in solitary confinement for long period of time (several weeks).

The majority (79%) of animals at WU experience mild discomfort. At WR, 50% of animals in 2022 experienced mild discomfort and 50% experienced moderate discomfort.

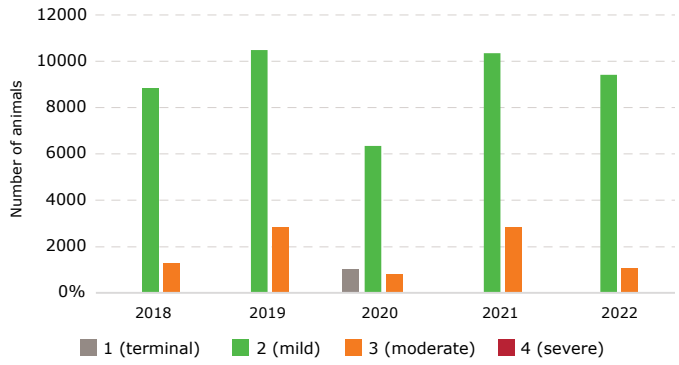
Discomfort as percentage WR



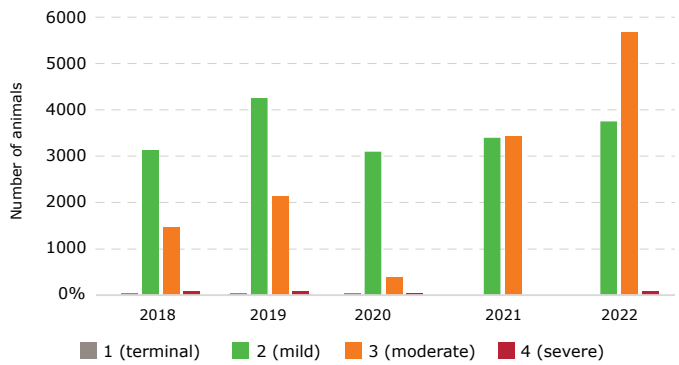
Discomfort as percentage WU



level of discomfort in absolute numbers WU



level of discomfort in absolute numbers WR



Fish stocks research

Wageningen Marine Research carries out research into fish stocks. The Data Collection Framework, an important piece of European legislation, consists of a number of ordinances and decisions that oblige Member States to collect data on the fisheries sector and fish stocks. This includes biological, economic and statistical data on the composition, size and distribution of fish stocks and fishing activities. The research largely focuses on fish from commercial fishing activities and research vessels.

Fish have to be killed to be able to determine the age distribution of fish stocks. Their age can be determined based on various anatomical features, such as the ear stones (otoliths), the scales, fin rays and the vertebrae, which form growth rings. Researchers also determine the sex, sexual maturity and fertility of the fish based on their sexual organs. Researchers may also examine the stomach contents and the presence of internal parasites. Tissues may also be taken for DNA and other analyses. The data collected in this way is used to support policy recommendations for the fisheries sector and ecosystem management.

The relatively large number of fish used as test animals and the level of discomfort is due to the nature of this research. Fish must be caught, tagged and recaptured. A fairly substantial catch is required in order to ensure that enough are recaptured and can then effectively inform policy recommendations regarding fisheries and ecosystem maintenance.

Looking ahead

We are aware that we are in the midst of a complex reality, where laws and regulations are constantly changing and being tightened, and society is becoming increasingly critical of the use of laboratory animals.

WUR wants to take this scrutiny and criticism on board in our discussions about how animals, including test animals, should be treated. We take these discussions seriously, as evidenced by the additional Ambitions and Guidelines discussed earlier in this report: we are doing more than the minimum required of us.

We are working hard to find validated alternatives to animal testing through research programmes like NLAS. We are also investing in developing our study programmes, and we aim to make our current and future researchers aware of the importance of the 3 Rs and of alternatives to animal testing.
But dialogues, looking for alternatives and adapting study programmes all takes time and does not deliver results in the very short term.

WUR conducts animal tests only when there really is no alternative, and we are persistent in our efforts to replace, reduce and refine. At the same time, we are aware of a growing number of questions related to sustainable food production, human and animal health, and how we interact with our environment. Animal tests are an important way of addressing these questions, and will remain so.

In the long term, our research with and for animals will contribute to building an understanding of how we as humans can live on this planet as harmoniously as possible with our environment. We therefore look to the future with confidence.

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