university of groningen

Animal studies:

Annual report 2019

The University of Groningen (UG) and the University Medical Center Groningen (UMCG) conduct animal experiments within the scope of research and teaching, because some important and relevant questions cannot be answered without the use of laboratory animals.

We are open about these activities and have created this website to show how we conduct animal studies and what we take into consideration in such testing. This is our contribution to the social debate about animal experiments in which anyone can form a considered opinion.

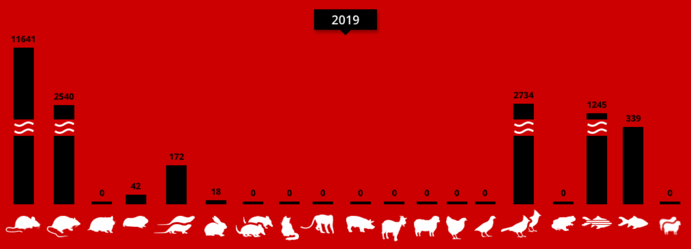
The Netherlands to spearhead alternatives for animal testing in 2025

The Netherlands has set itself the goal of becoming the worldwide leader in innovative research methods without animal experiments by 2025. As early as 1997, the Netherlands became a world leader in this area when it prohibited animal experiments for cosmetic products such as make-up, toothpaste, shampoo and deodorant. In 2004, the EU followed; since 2013, imports of such products from outside the EU have also been prohibited. In March 2016, State Secretary for Economic Affairs Martijn van Dam asked the *Nationaal Comité advies dierproevenbeleid* (NCad) to draft a transition scheme for the development of innovative animal-free research. This transition will be realized in collaboration with research organizations and private enterprise, and cover, for example, the statutory safety testing of chemical substances, allergy testing of new products, food ingredients, pesticides and vaccines. NCad believes that it should be possible to conduct such tests without animals being used by 2025. In other scientific domains, NCad also sees opportunities to gradually replace animal experiments with new techniques. In recent years, many alternative research methods have been developed that no longer require the use of animals. Examples include allergy tests on artificial human skin and a computer model of the heart used to investigate the effects of medication.

The University of Groningen supports initiatives to reduce the use of lab animals through innovative techniques and to become the world leader in such innovation by 2025. It is already promoting techniques such as the use of organoids (miniature organs cultivated from stem cells) and tissues and organs supplied by abattoirs as alternatives for lab animals. Nevertheless, future research will not be able to dispense with animal testing entirely. Particularly in medical biology, host-microbe interactions, neurobiology, physiology, pharmacy, immunology and behavioural biology, research into complex systems will remain essential. Organ-organ interactions and interactions between organisms and their environment are important elements of the research being conducted at the University of Groningen. At present, such interactions cannot be investigated adequately in animal-free systems.

Animal testing statistics

Within the UG, animal experiments are carried out for the benefit of fundamental and translational research, as well as for educational purposes. These experiments are carried out in the facility at the UMCG, or at the facility at FSE and there are also experiments taking place in the open field. In 2019, a total of 18,731 animal experiments were carried out, mainly on mice, rats and birds. In 2018, a total of 18,561 animal experiments were carried out. This shows a slight increase in 2019. In 2019, fewer experiments were carried out on mice and rats and more on zebra fish. The number of animal experiments shows an annual fluctuation due to available budgets and research capacity. The figure below shows an overview of the numbers of animal experiments that were carried out, categorized according to animal species, for the last 5 years.

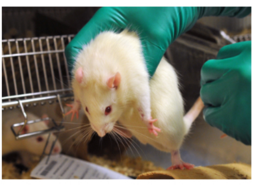


Total: 18,731

At the UG, the number of experiments on mice shows large annual fluctuations, whilst the number of experiments on rats continues to drop, as it has since 2013. This year we presented the Zebrafish data separate from the other fish strains for the first time.

The number of 'other' birds that are used in animal experiments has fluctuated for years and this is expected to remain the case for the foreseeable future. The Central Animal Facility (CDP) expanded its fish facility, which led to a relatively large increase in the number of fish used in 2019 compared to 2018. This is likely to increase over the coming years because there are now six different research groups that use the fish facility.

The number of animal experiments appears to be reasonably stable at present, following the introduction in 2014 of the new Experiments on Animals Act (WoD). This initially led to a decrease in the number of animal experiments, possibly due to the submission of new project applications and the processing of projects taking longer than before. The coming years will show whether these numbers are now stable.



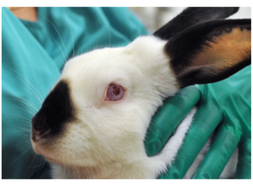
Why animal experiments?

Staying healthy while getting older (Healthy Ageing), adapting to changing circumstances (Adaptive Life) and creating a robust society (Sustainable Society) are policy spearheads of the UMCG and the UG. Many of our research programmes therefore focus on issues such as healthy ageing, Alzheimer's disease, diabetes and Parkinson's disease, which sometimes require animal testing. Animal experiments are also required to study ecological phenomena such as bird migration.

Animal experiments at the UG/UMCG

The University and the UMCG want their fundamental and applied research programmes to be among the best in the world. We wish to conduct the animal studies required to achieve this goal in the best possible manner, which means providing optimal care to lab animals and safeguarding of their welfare as well as optimal facilitation of the animal experimenters.

Our animal tests are conducted at the UMCG (65%) and the Faculty of Science and Engineering (FSE, 35%), where animal testing is concentrated in several research institutes.



▶ Behavioural & Physiological Ecology Group
Research into the behaviour of animals in their natural surroundings

▶ Conservation Ecology Group
Research into the impact of habitat changes on organisms

▶ Theoretical Research in Evolutionary Life Sciences (TRES)
Focus on theorical developments in evolutionary ecology, behavioural sciences and evolutionary systems biology

▶ Evolutionary Genetics, Development & Behaviour (EGDB)
Research into the proximate cause of phenotypic diversity and its ecological and evolutionary consequences

▶ Genomics Research in Ecology & Evolution in Nature (GREEN)
Research into ecological, evolutionary and conservationist issues in relation to biodiversity, ecosystems, living environment interactions, speciation, adaptation and plasticity

▶ Neurobiology
Research into the role of the brain in the capacity of animals and humans to adapt to challenges and opportunities in the environment

▶ Groningen Research Institute of Pharmacy (GRIP)
Fundamental and applied pharmaceutical research

▶ Groningen University Institute for Drug Exploration (GUIDE)
Development of new medication

▶ Health Research and Epidemiology (SHARE)
Fundamental and applied research into factors that help people to stay healthy while getting old (Healthy Ageing)

▶ European Research Insitute for the Biology of Ageing (ERIBA)
Fundamental research into factors causing ageing

▶ Biomaterials (W.J.Kolff Institute)
Applied research into biomaterials and implants

▶ Fundamental, Clinical and Translational Cancer Research (Cancer Research Center Groningen)
Fundamental and applied research into oncology and tumour development

Legislation and regulations

Animal studies are governed by strict legislation and regulations. Since 1977, the welfare of lab animals used in the Netherlands has been protected by the Wet op de dierproeven (Wod). To supplement this act, the Dierproevenbesluit (Animal Experiments Decree) was adopted in 1985. The principle underlying the act is the 'No, unless' principle: animal experiments are only allowed if there are no alternatives. If researchers can conduct a study by using a computer model or slaughterhouse material, for example, they will not be allowed to use animals for their experiments.

With the Wod, the Netherlands had good legislation governing the use of lab animals. There were major differences with other countries, however, including European member states. To achieve identical legislative standards – at least within the European Union – guidelines were drafted, which in the Netherlands led to a revision of the Wod in 2015. The current Wod defines an animal experiment as 'any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or teaching purposes, which may cause the animal a level of pain, suffering, anxiety or lasting harm equivalent to, or higher than, that caused by the introduction of a needle according to good veterinary practice'. Experiments conducted on animals without an endoskeleton, such as worms, snails and insects, are not covered by the Wod. The intention of the Wod is to protect lab animals in the Netherlands. One of its clauses stipulates, for example, that only qualified personnel are allowed to use lab animals and only within institutions that have a permit for such use.

In the old Experiments on Animals Act, two definitions were used for research involving wild animals: one covering their use in the laboratory and one covering animals living in nature. This distinction is no longer made in the newWod, which means that the same definition covers all animal testing, including research involving wild animals, whether in the laboratory or in their biotopes. It soon appeared that research involving wild animals in their biotopes was not covered in sufficient depth in the memorandum with the title 'Wanneer is er sprake van een dierproef in de zin van de wet?' ('When is an experiment an animal experiment under the Act?', in Dutch only) which was published on theCCDwebsite on 3 October 2016. A project group was therefore established with representatives from the relevant fields. In collaboration with theCCDand theNVWA, in 2017 the group published guidelines with the title 'Dierproeven met wilde dieren in hun biotoop' ('Animal experiments with wild animals in their biotopes', in Dutch only). UG researchers were involved in the formulation of these guidelines, which are used by UG researchers applying for and implementing animal experiments in nature.

Codes of Practice

Although legislation provides frameworks, it does not concern itself with details. For this reason, its specific interpretation may be unclear. Experts have therefore drafted several Codes of Practice covering various research fields: 'Animal experiments in Cancer Research' (1999), 'Immunization of Laboratory Animals' (2000) and 'Safeguarding the welfare of Lab Animals' (2001). Anyone working with lab animals must comply with these codes.
In addition, the Dierexperimentencommissie (DEC) of the UG has formulated internal guidelines to standardize University practices. These guidelines comprise the University's opinions about the discomfort codes, the choice of species and ethical considerations.

Animal experiments: from application to execution

CCD

The *Centrale Commissie Dierproeven* (CCD) is a national committee which makes decisions to grant or reject project licenses for experiments based on the research applications. On its website, the CCD publishes non-technical summaries of the licenses it has granted.

NCad is another important national committee. NCad's role is to bring about improvements in the application of the 3R principle and the ethical assessment thereof in scientific and applied research and in teaching activities, in order to minimize the use of laboratory animals both nationally and internationally.

In 2018, the IvD discussed 30 CCD applications and 27 were sent to the CCD, who submitted the projects for advice to the UG's Animal Ethics Committee (DEC).

Of the 27 projects sent, 23 were authorized by the CCD: 21 in 2018 and two in 2019. One project obtained partial authorization.

DEC

The UG has an impartial animal experiments committee (DEC–RUG) which assesses the use of lab animals under the auspices of the CCD, using the CCD's opinions and guidelines. It also abides by generally applicable viewpoints from the various codes of practice. The DEC–RUGmembership includes experts in laboratory animals and their protection, animal experiments, alternatives for such experiments and ethical assessment.

The DEC assesses all research proposals in the light of current legislation and regulations. It also weighs the benefits of animal experiments against the discomfort caused to the animals to be used.

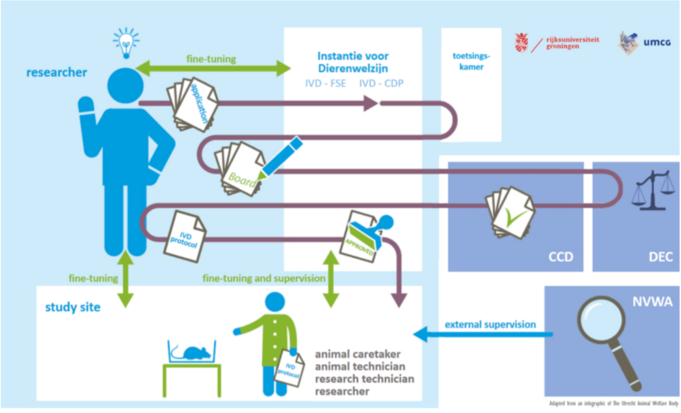
The intrinsic value of each animal is central to the decision whether an animal experiment is ethically acceptable or not. However, other considerations also play a role, for example an animal's psychological complexity (cf. primates), the societal status of a species based on factors such as social closeness (cats and dogs), historical value (agricultural animals) and social relationship (seals).

The UG and the UMCG do not have facilities for experiments conducted with primates, and the UG has formulated a separate point of view on this issue (in Dutch only).

IvD

An important change in the revised Wod is that institutions must combine their expertise concerning animal welfare in an *Instantie voor Dierenwelzijn* (IvD). The IvD assesses the animal welfare aspects of a research project that has previously been approved by the DEC and the CCD and ensures that it can be properly implemented. It also advises researchers about the application of the 3R principle and supervises the research preparations and the skills and training of the researchers involved.

The IvD membership includes a designated veterinarian, the animal facility's Location Supervisor, a scientist and, if necessary, an external expert such as a radiation specialist or biological safety officer.



Adapted from an infographic of The Utrecht Animal Welfare Body

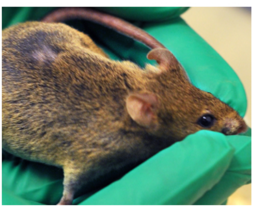
Article 14c of the Experiments on Animals Act lists the tasks of an Animal Welfare Body in five points (14c.1a to 1e). Article 14c.1c states that the IvD 'guarantees the establishment and review of internal procedures concerning monitoring, reporting and follow-up with regard to the wellbeing of the animals housed in the institution's animal housing facilities'. In other words, the Article states that the IvD organizes the laboratory animals' guaranteed wellbeing and produces a record of it.

For each IvD protocol, the UG's IvDs check whether the animal study will be carried out using animals that are caught in the wild. If so, the IvD checks whether the required flora and fauna dispensation has been obtained. This internal process will not change and continue to be used.

IvD platform

The national IvD platform represents all member IvDs in the Netherlands. They currently represent 45 IvDs, which is approximately 90% of all organizations. The IvD platform is in contact with government organizations such as the CCD, the Netherlands National Committee for the Protection of Animals used for Scientific Purposes (NCad) and the Netherlands Food and Consumer Product Safety Authority (NVWA). The IvD platform meets four times a year to discuss any current issues.

Another of the platform's important tasks is the promotion of contact between the IvDs. Last year, the platform organized two meetings that brought all IvDs together. The first meeting was the 'Severity Assessment' workshop, which was organized in collaboration with the NVWA and with input from the CCD. A team of representatives from the Federation of European Laboratory Animal Science Associations / European College of Laboratory Animal Medicine / European Society of Laboratory Animal Veterinarians (FELASA/ECLAM/ESLAV) Working Group and from the European Commission were also present to host the workshop. The second meeting took place during the 2018 Biotechnology days. At this meeting a workshop was hosted by the IvD Platform Working Group on Experimental Design & Statistical Analysis.



End of experiments

Adoption

The UG policy states that vertebrates – with the exception of mammals – that are being cared for at the UG in semi-natural circumstances and are no longer required for research may be eligible for adoption. An important condition is that animals can only be adopted by private persons. The permit holder does not allow the animals to be traded. No animals were available for adoption in 2017.

Euthanasia

In most cases adoption is not possible, for example because the brain and/or other organs and body parts are required for further study and analysis. In that case, the animals will be euthanized at the end of the experiment. This is a step which neither animal carers nor researchers take lightly. The most common euthanasia procedure is one which the animals hardly notice. They are placed in a box containing a mixture of oxygen (O_2) and carbon dioxide (CO_2). Then the CO_2 concentration is slowly increased, causing the animals to gradually lose consciousness and then pass away peacefully. Sometimes the nature of the experiment requires a different euthanasia procedure. In such cases, too, the method chosen must result in the least possible discomfort for the animal. In some cases, animals develop complications over the course of an experiment, which may lead them to suffer more than expected. Researchers will then apply the principle of the humane end point. They will remove the animal from the experiment when its suffering threatens to become unacceptable and then euthanize it to prevent further suffering.

Aims of animal experiments

By far the most animal experiments were carried out to help answer a scientific question. The figure below indicates what these questions entailed. In addition to answering scientific questions, animal experiments were also conducted within the scope of teaching and training, involving, for example, students and animal technicians.

3.120 endocrinology and metabolism

615 circulation and lymphoid organs

338 multi-system research

1.323 cancer research

164 sensory organs

3.873 nervous system

Animal experiments for the benefit of fundamental research subdivided by goal

12 respiratory system

0 musculoskeletal system

784 immune system

14 urogenital system

3.284 ethology, animal behaviour, animal biology

1.380 gastrointestinal tract (incl. liver)

3.033

Applied and translational research

35

Not used in animal experiments

14.907

Research

591

Education

198

Protection of animal species

Discomfort

Lab animals will always experience some degree of discomfort. The revised Wod divides discomfort into four categories. Discomfort need not take the form of pain; stress and anxiety are also regarded as discomfort. The table below shows the degrees of discomfort and the percentages of animals involved in 2019.

4%	68%	27%	1%	0%
Termination	**Mild discomfort**	**Moderate discomfort**	**Substantial discomfort**	**More than substantial discomfort**

Breeding efficiency

The UG and the UMCG breed animals themselves, particularly (transgenic) mice and rats. Not all animals bred are used in experiments. In 2017, 40,039 animals were bred, 28,609 of which (about 58%) were not used for experimental purposes. These animals are referred to as ‘surplus animals’ or ‘breeding surplus’. National and international organizations and governments acknowledge that the large number of surplus animals is a problem.

Unfortunately, such surpluses are unavoidable. To obtain reliable research findings, animals in an experiment must be as identical as possible. They should have the same age and sex, for example, or have been born under identical circumstances. Animals also differ in genetic properties, some of which may be undesirable. For an experiment involving 60 identical transgenic mice, for example, as many as 170 mice may have to be bred. For more information, consult the website of the Stichting Informatie Dierproeven (Animal Experiments Information Foundation, in Dutch only). In addition, a substantial percentage of animals bred is required to preserve unique or valuable breeding lines.

Reducing the number of animals that are bred, but not used in experiments is high on the UG’s list of priorities. In 2018, it was still the case that approximately a quarter of the animals bred were supplied for experiments (a few percent more than in 2017). The UG is strongly aware that the number of animals bred and not used is still too high and that a continued effort must be made to reduce the number of animals that are killed without being used.To do so, the UG has started to offer cryopreservation of breeding lines that are no longer actively used for animal experiments.

Cryopreservation

Rather than maintaining a breeding line that is not needed for some time with live animals, egg cells or sperm are frozen by using a technique called cryopreservation. If the breeding line is needed again, a fertilized egg cell is introduced into a pseudopregnant female. This means that no animals are required to maintain the line in the interim period.
However, freezing egg cells and sperm involves a complicated procedure, with many frozen embryos not being viable, for example. The UG and the UMCG regard cryopreservation as an important technique for reducing the breeding surplus, with the main focus on sperm cryopreservation. This is a highly efficient method for freezing a breeding line, since it requires only two male mice. Stopping breeding lines temporarily through cryopreservation reduces the number of mice by about 200 per breeding line.

Replacement, Reduction, Refinement

The UG and the UMCG apply the 3R principle to research and teaching involving laboratory animals: replacement and reduction of the number of animals and refinement of the experiments in which they are used. Essentially, this means that we use as few animals as possible and conduct animal-free experiments whenever possible. Furthermore, we try to minimize the discomfort experienced by the animals. The Animal Welfare Committee (IvD) helps researchers to put these guidelines into practice.

Replacement

Researchers are only allowed to conduct an animal experiment if there are no other options. Where possible, we use alternatives to animal experiments in teaching and research, replacing laboratory animals with invertebrates, cells, tissues, computer simulations, video training or slaughterhouse material.

Reduction

Efforts must be made to reduce the number of animals required in each experiment through a research design specifying the minimum number of animals necessary to achieve reliable findings. This can be achieved, for example, by using standard strains so that the results are more comparable or by conducting a pilot study first.

Sometimes lab animals can be used again after the original experiment, in a follow-up or unrelated experiment or in a teaching activity. In 2019, 2% of animals was used again.

Refinement

Researchers, animal carers, animal technicians and designated veterinarians are always trying to refine all aspects of animal use and animal welfare. Optimum accommodation and adequate application of research techniques and anaesthesiology should minimize the animals discomfort. Social animals such as rats, for example, are kept in groups, which reduces their stress levels.

By refining animal experiments, we improve the animals’ welfare, which is not only good for them but benefits the quality of research too.

Applying the 3R principle in teaching activities involving lab animals

Training

The *Centrale Dienst Proefdieren* (CDP) applies the 3R principle as much as possible when lab animals are used for teaching purposes. When inexperienced students are first introduced to a technique, synthetic materials are used as much as possible. Students learning to suture, for example, first practice on a piece of chamois leather.

Microsurgical techniques are first practised using a piece of latex glove under the microscope and then on artificial vessels. If the students' hand-eye coordination is sufficiently developed, they are allowed to continue with live rats. Synthetic materials are thus used whenever possible. Ultimately, however, the technique to be mastered must be practised in a live organism, since a living animal presents students with a system that is too complex to mimic with synthetic materials. To further reduce the number of animals used, instruction videos have been produced for all relevant biotechnical procedures covered during student training, so that no animals have to be used to demonstrate the techniques. Animals used in teaching are always anaesthetized before an invasive surgical procedure and are euthanized before the anaesthesia wears off, to prevent unnecessary discomfort. By thoroughly training the staff involved in animal experiments, the CDP aims to improve the quality of animal experiments and the animals' welfare.

Anatomy practical

All Bachelor students of Biology take an anatomy and physiology practical involving the dissection of a rat. Until 2015, these rats were always euthanized shortly before the start of the practical and presented untreated to the students because this procedure results in the best specimens. In frozen and subsequently thawed specimens, certain essential structures proved difficult to see. Over the year, there are sufficient surplus animals available from breeding and invasive and non-invasive experiments to meet the needs of this practical. From the perspective of animal welfare, however, it is undesirable to keep these animals alive until the start of the practical. This is also uneconomical. For this reason, until 2015, the rats used were purchased from a commercial breeder. Despite the fact that almost all these animals were surplus animals from their breeding lines, this situation was less than ideal, not least because of the stress caused to the animals, for example during transport.

For these reasons, 2016 witnessed the start of a highly successful pilot in which surplus rats from our own breeding programme and experiments were embalmed. These embalmed rats proved highly useful in the practical because all the important structures were preserved well, which was not the case with the frozen specimens.

To embalm these rats, the Fix for Life method developed by Leiden University Medical Centre was used. This method employs an embalming fluid which is (virtually) free of the toxic and irritant substances such as formaldehyde and phenol that are commonly used to preserve tissues. This makes the method extremely suitable for teaching purposes. Another advantage is that the embalming fluid has a less offensive smell.

Embalming the rats has proven a win-win situation. First, rats no longer have to be purchased and transported, and our own surplus animals can now serve a useful purpose. At the same time, the specimens have proven extremely suitable for the practical, and their use is less taxing on students.

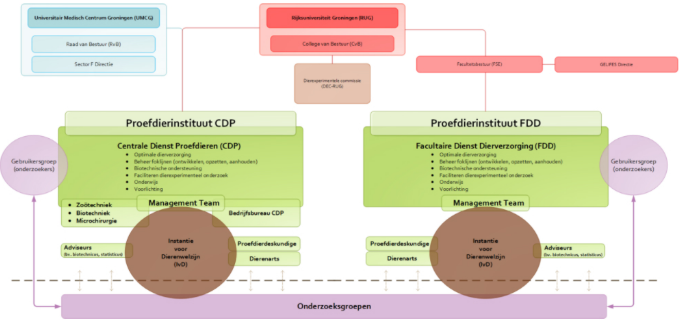
Organization and facilities

To guarantee optimum animal care and effective research, two modern animal experiment facilities have been set up: CDP at the UMCG and the *Facultaire Dienst Dierverzorging* (FDD) at the Linnaeusborg.

All animal studies at the UG and the UMCG are conducted either in nature or in one of the laboratories with special animal testing facilities. We take the utmost care to provide the best possible accommodations for lab animals, since they will live out almost their entire lives there. Providing accommodation thus involves more than simply meeting the statutory requirements. The CDP and the FDD have been completely renovated in 2009 and 2011, respectively, and are now among the most modern facilities in Europe. The temperature, lighting and atmospheric humidity in the animal quarters can be precisely controlled.

Inspections by the NVWA

The NVWA carried out two inspections in 2018 (one site visit and one office inspection) and all was in order.


Universitair Medisch Centrum Groningen (UMCG)
Raad van Bestuur (RvB)
Sector F Directie
Rijksuniversiteit Groningen (RUG)
College van Bestuur (CvB)
Dierexperimentele commissie (DEC-RUG)
Faculteitsbestuur (FSE)
GELIFES Directie
Proefdierinstituut CDP
Centrale Dienst Proefdieren (CDP)
Optimale dierverzorging
Beheer foklijnen (ontwikkelen, opzetten, aanhouden)
Biotechnische ondersteuning
Faciliteren dierexperimenteel onderzoek
Onderwijs
Voorlichting
Gebruikersgroep (onderzoekers)
Zoötechniek
Biotechniek
Microchirurgie
Management Team
Bedrijfsbureau CDP
Adviseurs (bv. biotechnicus, statisticus)
Instantie voor Dierenwelzijn (IvD)
Proefdierdeskundige
Dierenarts
Proefdierinstituut FDD
Facultaire Dienst Dierverzorging (FDD)
Optimale dierverzorging
Beheer foklijnen (ontwikkelen, opzetten, aanhouden)
Biotechnische ondersteuning
Faciliteren dierexperimenteel onderzoek
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Proefdierdeskundige
Dierenarts
Instantie voor Dierenwelzijn (IvD)
Adviseurs (bv. biotechnicus, statisticus)
Onderzoeksgroepen

Fish facility

In 2015, two of our researchers stated that they wanted to use animal models with two species of tropical freshwater fish: zebrafish (*Danio rerio*) and killifish (*Nothobranchius furzeri*). Although the CDP already had the expertise to breed, house and care for zebrafish, it had no experience with the other species.
To obtain this expertise, internships were organized at a foreign killifish laboratory and collaboration with Dutch colleagues was sought. The fish facility is now running smoothly. For an explanation of the experiments involving killifish, see the interview with researcher Dr Berezikov.

About the University of Groningen

The University of Groningen is a research university with a global outlook, deeply rooted in Groningen, City of Talent. The UG is in the top 100 of several important ranking lists. It is very popular with its 30,000 students and staff (5250 FTE) from the Netherlands and abroad, who are encouraged to make the most of their abilities. Talent is nurtured, and the keyword is quality. The University is committed to actively cooperating with its partners in society, with a special focus on its research themes Healthy Ageing, Energy and Sustainable Society.

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