



K O N I N K L I J K E N E D E R L A N D S E
A K A D E M I E V A N W E T E N S C H A P P E N

Report on the Use of Animals in Research

Royal Netherlands Academy of
Arts and Sciences
Organisation for National Research
Institutes
2011- 2012

Amsterdam, September 2013



2013 Royal Netherlands Academy of Arts and Sciences (KNAW)

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Translation: Balance, Amsterdam/Maastricht

ISBN 978-90-6984-675-0

Available as a PDF at www.knaw.nl

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INTRODUCTION

Three of the Academy's seventeen institutes use animals in research on physiological mechanisms in humans and animals. Using mice, for example, helps us to understand how intestinal cancer metastasizes, while the eggs of zebrafish can help to study how the heart, bones, and blood vessels are formed. We carry out research on neurological disorders by studying rhesus monkeys, and great tits can help us understand how animals adapt to the climate and to human disturbances of their living environment.

Using animals in research causes tensions as regards ethics. On the one hand, we want to combat disease and human suffering, but on the other we wish to reduce the use of animals in research and preferably to dispense with it entirely.

The Royal Netherlands Academy of Sciences adopts an extremely careful approach to experiments using animals, avoiding them whenever that is at all possible. Our scientists submit plans for animal experiments to a special committee which assesses whether each experiment is actually necessary, or whether the study concerned can be carried out in a less drastic manner, or using other animals.

In some respects, the Academy goes beyond what is in fact required by law. For example, researchers submit plans for experiments to the committee even when the law does not require them to be assessed. The Academy has also set up a special course on the use of animals that focuses not only on mice and rats and also on birds, fish, and wild animals. In addition, we have set up a fund which our researchers can utilise when they have ideas about how research can be carried out more effectively with fewer animals, or causing less distress to them.

The present biennial report is a survey of the scientific research carried out by the Academy's institutes using animals. Its aim is to render an account and to provide information. The report is intended not only for those directly involved – for example policymakers, organisations opposed to experiments using animals, and researchers – but also to anyone else who may be interested. With that in mind, we have made every attempt to avoid using jargon.

Prof. Theo Mulder
Director of Research

1. POLICY

The aim of the experiments using animals at the Academy's research institutes is to generate knowledge about the mechanisms of fundamental biological processes. Three Academy institutes carry out experiments using animals:

- The Hubrecht Institute for Developmental Biology and Stem Cell Research studies processes in the human body. The main questions addressed concern how processes go wrong and how they can lead to illness and death. Research at the institute forms the basis for new methods of treatment.
- The Netherlands Institute for Neuroscience focuses on the brain and the visual system. Its researchers study the development, ageing and adaptability of the brain, often in connection with questions arising in clinical practice.
- The Netherlands Institute of Ecology aims to acquire a better understanding of how animals behave in their natural environment, how they adapt to a changing world, and what physiological mechanisms underlie their behaviour.

Position of the Academy

In 2010, the Academy published its position regarding experiments using animals and the importance of such experiments. To summarise, the Academy believes that our knowledge of nature, health, and illness is to a large extent based on research using animals. The Academy considers that experiments using animals are necessary for biological and biomedical research because in many cases alternatives that are of – at least – equivalent value are unavailable. But that is not simply to say that the Academy is in favour of experiments using animals. In fact, it believes that everything possible must be done to reduce the number of such experiments and to minimise the resulting distress for the animals used.

Replacement, Reduction, Refinement

The Academy's policy regarding the use of animals in research focuses on the "Three R's": replacement, reduction, and refinement. Replacement means looking for alternatives. Reduction means trying to use fewer animals, for example by applying more efficient research methods. Refinement refers to reducing the distress suffered by these animals, for example by housing and caring for them better. The Academy provides EUR 100,000 from its "Three R's Fund" to finance measures aimed at replacement, reduction, and refinement. (See also the sections on "Better housing for birds" and "Human retina".

Better housing for birds

The Three R's Fund has enabled the Netherlands Institute of Ecology to improve the housing provided for the water birds that it studies. Animals competing for their food – passerine birds and rodents – can no longer gain access to the birds' food. The water intake has been relocated to the middle of the artificial ditch, meaning that the latter can now be cleaned more easily. The various species of birds are now separated into different compartments, and the birds are now able to access an outside area where they can graze.

Human retina

The Netherlands Institute for Neuroscience used a grant from the Three R's Fund to purchase a special microscope. This enables the researchers to dissect and prepare human donor retinas and cultivate them for research purposes. The cultivated retinas remain in good condition for about two weeks, thus allowing the researchers to carry out physiological experiments with them. Previously, it was necessary to use the retinas of zebrafish or mice.

Careful selection of animal species

Careful decisions are taken at the three institutes as to whether using animals is in fact necessary for the research carried out there, and if so which species. The basic principle is that an experiment should not be carried out with a vertebrate if an invertebrate can also be used.

Openness and security

The Academy strives for greater openness regarding experiments using animals. Together with the Association of Universities in the Netherlands (VSNU) and the Netherlands Federation of University Medical Centres (NFU), it has therefore drawn up a Code on Openness with Regard to Experiments on Animals [Code Openheid Dierproeven]. Institutions that have signed up to the Code must account publicly for their use of animals in research. The present Report on the Use of Animals in Research is part of that approach. The provision of education and information is also obligatory. However, openness about experiments using animals involves various dilemmas. Being too open can put our researchers at risk, and we cannot always provide full information because some research data is sensitive from the point of view of competition. We are therefore constantly seeking a balance between openness, confidentiality, and security.

Own more thorough course

Researchers who work with animals must have studied basic biological subjects for a minimum of five hundred hours. They must also have taken the special course on the use of animals in research, at which they learn to adopt a critical attitude regarding the design of their research. The courses that are organised elsewhere in the Netherlands focus primarily on mice and rats and hardly at all on birds, fish, and wild animals. Because the Academy's institutes also use these species – in captivity or in the wild – we developed a course in 2009 that deals with all the species that the institutes use. The course has been recognised at national level. The Academy is now offering it for researchers and staff of non-Academy institutes.

More information:

- Academy page on experiments using animals: <https://www.knaw.nl/dierproeven>
- Academy position regarding experiments using animals: <https://www.knaw.nl/publieksstandpunt-dierproeven.pdf>

2. EXAMPLES

This section outlines the research using animals at the three Academy institutes that use such animals. An example of a particular line of research has been given for each institute.

Hubrecht Institute for Developmental Biology and Stem Cell Research

The Hubrecht Institute studies how embryos develop and how organs grow. It uses mice, rats, zebrafish, frogs, insects, nematodes, and flatworms in its research. The institute studies diseases such as cancer and diabetes, in which organs often display uncontrolled growth or have been damaged. Amongst other results, the research generates knowledge on how to treat these conditions. The researchers study stem cells because these can develop into specific tissues, for example for the heart, brain, intestines, and pancreas. Doctors believe that stem cells have potential for use in treatment, but before stem cell therapy can be applied we need to know how to control the growth and specialisation of these cells.

Using genetically modified mice, the Hubrecht Institute investigates the origin of intestinal cancer and metastases (see the box on 'Filming how cancer metastasises'). Researchers attempt to cure diabetic mice by injecting them with pancreatic stem cells. Rats are used to make 'genetic knock-outs': the animals lack one or more specific genetic characteristics; this enables the researchers to study that characteristic and to attempt to correct the defect, for example by means of nutrition, medication, or supplements. By using zebrafish – and particularly their eggs – the biologists can study the development of the heart, bones, and blood vessels. They also investigate the origin of diseases associated with the development of these organs and how they can be prevented or cured. The institute also uses frogs' eggs to produce extracts with which researchers can study the mechanism underlying DNA repair.

Filming how cancer metastasises

Jacco van Rheenen, a researcher at the Hubrecht Institute, explains: 'Currently, the only treatment option for cancer patients with metastases is chemotherapy. Unfortunately, that has many side-effects and the metastases frequently reoccur. Our aim is to develop new and improved methods of treatment. In order to do so, we need to understand how a tumour metastasises and why metastases are sometimes resistant to chemotherapy. We use advanced microscopic techniques to visualise the process of metastasis in a tumour. We take photos and make films of tumour cells in living mice.

Our research has revealed that the movement of cells is extremely important in the development and metastasis of tumours. We have also been able to show that the cells in a tumour are not all equal and that there is in fact a hierarchy in which only a few cells – the cancer stem cells – are responsible for the growth of a tumour. In the near future, we intend investigating whether these cancer stem cells are also responsible for metastasis and resistance to chemotherapy.

We have also shown that tumour cells are not independent units and that they share genetic material and proteins both locally and remotely. This type of communication is perhaps important

in the formation of tumours and metastases. We intend doing more research on this. We are also studying how the immune system influences the development of cancer. It was thought that the immune system offers protection against this but our microscope experiments have shown that some immune cells actually stimulate the process of metastasis. This information can help develop new methods of treatment in which immune cells play a role. We would never have been able to carry out our research without using animals.'

Netherlands Institute for Neuroscience

The Netherlands Institute for Neuroscience (NIN) carries out fundamental research on how the brain functions and on diseases that disrupt its functioning. The knowledge generated can help in the short or medium term to detect and combat brain disorders. The researchers focus particularly on the processes in the brains of healthy animals. They also study common brain diseases and their causes.

Researchers use rats, for example, to investigate the effects and treatment of paraplegia and multiple sclerosis. They also use mice and rats to study Parkinson's and Alzheimer's diseases (see the box on "Memory and Alzheimer's disease"). They measure, for example, the activity of nerve cells and investigate which proteins play a role in nerve cells that are functioning normally. They also study how the brains of rats control their hormonal system.

Using zebrafish, mice and rats, researchers at the institute investigate how light entering the eye is converted into signals that are transmitted to the brain. This helps them to understand how the brain processes signals and they also study whether blindness can be prevented or repaired by means of medication or prostheses.

With the aid of a small number of rhesus monkeys, researchers study thought processes and attention deficit; this plays a role in patients suffering from Alzheimer's disease or ADHD. The researchers also use rats to improve the technology for deep brain stimulation. This technology is already being used for some psychiatric patients and Parkinson's disease sufferers; a pacemaker in the brain reduces or boosts brain activity. This approach enables researchers to discover new locations within the brain where this technology can be utilised.

The Netherlands Institute for Neuroscience uses as few animals as possible in its research. In some cases, however, using them may be unavoidable, given the enormous consequences that brain diseases have for patients suffering from them.

All the animals used by the Academy's institutes are very well looked after. The monkeys are housed in large state-of-the-art cages, and as far as possible in pairs because they prefer this. All animals used have been born and reared in captivity; they have therefore not been captured in the wild.

Memory and Alzheimer's disease

Helmut Kessels, a researcher at the Netherlands Institute for Neuroscience, explains: "Humans and animals can store a great deal of information in their brain. When we learn something, some connections within the brain become stronger and others weaker. We use mice to study these orchestrated changes. We allow them to explore a new environment and we then examine the changes that take place in the brain connections. By blocking these changes, we can study how the blockage affects memory."

We know that Alzheimer's disease affects the connections within the brain, and also that a protein – beta amyloid – is one of the causes. Thanks to research on animals, we have shown that brain connections that are actively used are not affected by this protein. That might well explain why, on average, Alzheimer's patients who frequently engage in intellectual activities develop memory problems later than those who do not. We hope that our research will form the basis for developing a therapy that can delay or alleviate Alzheimer's disease."

Netherlands Institute of Ecology

Researchers at the Netherlands Institute of Ecology (NIOO) focus on the permanent interaction between animals, plants, and their environment. Amongst other things, the institute's researchers study how plants and animals adapt, and they investigate the variation within populations.

NIOO researchers study animals in their natural habitat and it is therefore important that the research has only a minimum impact on them. Researchers carry out behavioural tests, for example, or they relocate the animals temporarily. In some cases, they extract a few drops of blood. The institute's researchers make no use of genetically modified animals.

The institute has been monitoring nest boxes of great tits since 1955. Researchers count the eggs, ring the nestlings, and read the rings worn by the parent birds. This information is used, for example, to study the effects of ageing (see the box on "The great tits of Vlieland").

In some cases, the great tits are equipped with a tiny transmitter. A number of Bewick's swans have been equipped with a GPS data logger that tells the researchers where they are at a given time. This enables the researchers to determine whether birds that migrate to their breeding ground early in the season produce more young than those that do so later. When the birds are having the transmitter fitted, the researchers also take blood samples and a few feathers from them. This allows them to determine what the birds have been eating and where they have lived.

The Netherlands Institute of Ecology has a number of special aviaries where two lines of great tits are being bred. Just like people, the birds differ as regards their personality. The institute has both "bold" and "timid" birds. Researchers study how personality affects the species. They can also simulate climate change by adjusting the temperature, lighting, and food supply in the aviaries. The researchers observe whether the birds adapt, for example by starting to lay their eggs earlier. At the end of the experiment, the birds are released after undergoing a special adjustment programme. They appear to cope well with life in wild.

The great tits of Vlieland

Arie van Noordwijk, a researcher at the Netherlands Institute of Ecology, explains: "It was always thought that in nature ageing played hardly any role. Animals were thought to die before they really became old. We have shown, however, that ageing is indeed relevant in natural populations. Part of our research involves studying telomeres – the extremities of the DNA – in great tits. We know that some people and animals have longer telomeres than their peers and that, on average, they live longer. We also know that the telomeres become shorter in the course of someone's life. That already happens due to exertion, for example, and is not normally anything to worry about. We have been monitoring great tits on the Dutch island of Vlieland since as far back as 1955, and we now have a more or less complete family tree for them. The nice thing about the great

tits on Vlieland is that there are two subpopulations. Each year, far more representatives of one subpopulation survive than of the other. Once or twice a year, we extract a few drops of blood from the birds, allowing us to measure the length of the telomeres. Because there are two subpopulations, we can see whether and how the length of the telomeres is associated with the birds' survival, and what role is played by heredity. The results of our research also help to understand ageing processes and survival in humans.

3. SUPERVISION AND MONITORING

The Academy's Committee on Experiments using Animals checks every research plan to determine whether the use of an animal is justified by the scientific and public interest of the research. The Dutch Experiments on Animals Act [Wet op de dierproeven] prohibits experiments using animals if alternatives are available, if other – often “lower” – animals can be used, or if the experiment can be carried out in a manner that causes less distress to the animals concerned. The Academy's Committee on Experiments using Animals sends an annual report on its work to the Dutch Food and Consumer Product Safety Authority [Nederlandse Voedsel en Waren Autoriteit], which then publishes a national overview.

Internal monitoring

The Academy has two experts on the use of animals on its staff. Their task is to monitor animal welfare, to check whether each experiment using animals is performed as described in the research plan, and to check whether the conditions set by the Committee on Experiments using Animals are being complied with. The two experts also update the relevant records and make recommendations to the Committee on Experiments using Animals. They also have a monthly consultation meeting with the Academy's Director of Research; it is he who is legally the holder of the permit for experiments using animals.

Committee on Experiments using Animals

The Academy's Committee on Experiments using Animals holds monthly meetings. Its members are experts in the fields of experiments using animals, protection for these animals, alternatives, and ethics.

The Committee consists of:

- an independent chairperson not employed by the Academy and not involved in the experiments using animals;
- an independent expert in the field of ethics who is not involved in the experiments using animals;
- an independent expert on the use of animals in research and their protection, alternatives, and ethics;
- a representative of the Hubrecht Institute for Developmental Biology and Stem Cell Research;
- two representatives of the Netherlands Institute for Neuroscience (each monitoring a different scientific discipline);
- a representative of the Netherlands Institute of Ecology;
- a representative of the institutes regarding the use of animals in research and their protection (in the years covered by the present report, this was a member of the staff of the Netherlands Institute for Neuroscience).

The representative of a given institute is not permitted to vote on research plans submitted by his/her own institute.

Procedure and Exceptions

A lengthy procedure has already taken place before the Committee on Experiments using Animals deals with a research plan. The researcher first discusses the experiment with his/her superior. He/she then produces a plan setting out the purpose of the experiment, how it is to be performed, and why it is of scientific or public importance. He/she also explains why a specific animal is required for the research. The Academy's experts on the use of animals then discuss the plan with the researcher. The research plan is then submitted to the Committee on Experiments using Animals. The Committee studies the plan, checks it against the relevant legislation, and submits its recommendations to the Academy official who is the permit holder. The latter has the final say. If the Committee's recommendation is negative, the researcher can revise the research plan and resubmit it to the Committee; he/she may perhaps also explain it in person.

There are a few exceptions to this procedure. In some cases, a research plan need not be considered by the full Committee, for example if only a very low level of distress is involved. Repeat experiments may also only need to be dealt with by a small number of members of the Committee if they are carried out according to a fixed protocol and have already been assessed by the full Committee. Experiments involving monkeys must always be assessed by the full Committee.

The Committee holds its meetings at the three institutes alternately and regularly invites the institutes' research coordinators to explain their research and the context within which it takes place.

Much of the research involving animals that is carried out by the Academy's institutes is not subject to the provisions of the Experiments on Animals Act, for example the production of certain types of transgenic zebrafish, and research using fish eggs and fish embryos; counting great tit nestlings is not subject to the Act either. Nevertheless, the Academy has decided that this research must also be discussed with the experts on the use of animals. If there is even the slightest doubt – for example regarding the risk of distress – then the research plan must be submitted to the Committee on Experiments using Animals.

External Supervision

Inspectors from the Dutch Food and Consumer Product Safety Authority regularly carry out inspections of the Academy's institutes, with and without prior notice being given. The inspectors check whether the experiments involving animals are being carried out according to the plan. They also talk to the permit holder and to the experts on the use of animals, and they clarify the regulations. In addition, they check whether the institute's staff are looking after the animals properly and whether the right records are being kept. They also check the animals' accommodation. The inspectors were very pleased, for example, with the new facilities for housing water birds set up by the Netherlands Institute of Ecology.

4. FIGURES

The tables below set out the main figures for the past four years. During that period, there was an increase in the number of research plans submitted but a reduction in the distress suffered by the animals. The growth in the number of research plans was due to the increase in size of the institutes. The reduction in distress was because of improved methods of analysis.

More research plans

	Hubrecht Instituut	Netherlands Institute for Neuroscience	Netherlands Institute of Ecology	Total	Positive recommendation immediately	Positive after revision	Negative/ withdrawn
2009	41	68	12	121	104	13	4
2010	41	65	12	118	101	11	6
2011	65	96	10	171	155	12	4
2012	81	107	19	207	177	19	2

Table 1: Research plans submitted to the Committee on Experiments using Animals. The Hubrecht Institute for Developmental Biology and Stem Cell Research took over a department of the Utrecht University Medical Centre and now has about a third more staff. The institutes also receive a large number of European research grants.

** The figures to the right of the total do not add up to 207 (177+19+2=198). This is because 9 decisions would not be issued until 2013.*

Fewer fish

	Mouse	Rat	Fish	Bird	Rhesus monkey	Other*	Total
2009	6000	1800	2500	4000	6	4	14500
2010	6500	1500	2000	5000	2		15000
2011	8500	1500	1500	8500	7		20000
2012	9000	1500	750	6500	4	5	17500

Table 2: Annual number of experiments using animals (large numbers have been rounded off). The same animal may sometimes be used for more than one experiment. If an animal is used for two experiments, for example, it is listed twice in the table. The number of fish used has fallen steadily; this is because researchers are increasingly using only their eggs. (See also the examples in Section 2.)

** In 2009, four rabbits were used. In 2012, five frogs were used.*

Less distress

	Slight	Slight/ moderate	Moderate	Moderate/ serious	Total
2009	4500	6000	2000	2000	14500
2010	5000	7500	1500	1000	15000
2011	8000	8500	2500	1000	20000
2012	4500	8500	3500	1000	17500

Table 3: Extent of distress (rounded off). There has been a shift from the “moderate/serious” category to the “moderate” category. In 2009, 2010, 2011 and 2012, the Academy’s institutes did not carry out any research involving serious or very serious distress.

Number of inspections constant

	Prior notice given	No prior notice given	Total
2009	6	1	7
2010	1	3	4
2011	7	5	12
2012	4	5	9

Table 4: Inspections by the Dutch Food and Consumer Product Safety Authority. The number of inspections has remained more or less constant.

