



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 2013 and 2014

by the three Academy institutes that
work with laboratory animals

- Hubrecht Institute for Developmental Biology and Stem Cell Research
 - Netherlands Institute for Neuroscience
 - Netherlands Institute of Ecology

Amsterdam, July 2015



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Royal Netherlands Academy of Arts and Sciences (KNAW)

P.O. Box 19121, 1000 GC Amsterdam

T +31 (0)20 551 0700

F +31 (0)20 620 4941

knaw@knaw.nl

www.knaw.nl

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INTRODUCTION

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

The Academy avoids animal experiments whenever that is at all possible, and in some respects it goes beyond what is in fact required by law. For example, our researchers submit plans for animal experiments for assessment by a specially appointed committee,¹ even though that is not a legal requirement. The Academy also runs a special course on laboratory animal science that focuses not only on mice and rats (as legally mandated) but 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 laboratory animals, or causing less distress to them.

The present biennial report is a survey of research involving laboratory animals carried out by the Academy's institutes. The report is intended not only for those directly involved – for example policymakers, organisations opposed to animal experiments, and researchers – but also to anyone else who may be interested.

New compared to the previous report is a list of frequently asked questions, with answers. The FAQs answer many of the questions posed in the Transparency Benchmark on research using animals that has been drawn up by the Dutch Society for the Replacement of Animal Testing [*Stichting Proefdiervrij*].

We hope that this report will also serve to demonstrate that the Academy is very much in favour of openness regarding animal experiments.

Prof. Theo Mulder

Academy's Director of Research and official permit-holder for animal experiments

¹ See Section 3.

1. POLICY

The aim of animal experiments at the Academy's research institutes is to generate knowledge about fundamental biological processes. Three Academy institutes make use of laboratory 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.
- 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.

More information about animal experiments and the Academy is available on a special website: www.knaw.nl/nl/thematisch/ethiek/dierproeven/overzicht. Further information about the research carried out at the institutes is given in Section 2 '*The three institutes and their research*'.

Position of the Academy

In 2010, the Academy published its opinion on research involving laboratory animals and the importance of such research (see also www.knaw.nl/publieksstandpunt-dierproeven.pdf). That opinion still applies. To summarise: the Academy considers that animal experiments are necessary for biological and biomedical research because in many cases alternatives that are of equivalent value are unavailable. But that is not simply to say that the Academy is in favour of animal experiments. Everything possible must be done to reduce the number of laboratory animals used, and to minimise the distress that they experience.

Replacement, Reduction, Refinement

The Academy's policy regarding laboratory animals focuses on the 'Three R's': replacement, reduction, and refinement. Replacement means looking for alternatives, for example a different species of animal or a cell line or a system that does not involve animals. Reduction means that the Academy tries to use fewer animals, for example by applying a different experimental design. Refinement refers to reducing the distress suffered by laboratory animals, for example through the use of refined techniques or equipment, or by housing or caring for them better. The Academy provides EUR 100,000 annually from its 'Three R's Fund' to finance measures aimed at replacement, reduction, and refinement. Some examples of what this money has been used for are given in the boxes '*Environmental enrichment for mice*' and '*Transponders for bird research*'.

Environmental enrichment for mice

The Netherlands Institute for Neuroscience has used money from the Three R's Fund to investigate whether mice have a fitter brain if they are housed in an enriched living environment. We use 'Marlau cages' where mice can pass through tunnels and stairways via several spaces, have to go through a maze to find their food, and can interact more socially. This is a much more natural environment for the mice than a bare and uninteresting cage.

Transponders for bird research

The Animal Ecology department of the Netherlands Institute of Ecology carries out research on birds, especially on species that breed in nest boxes. Much of this research involves measuring the efforts of the parent birds when raising their young. It is relatively easy to investigate those efforts by using a 'transponder'. This is a chip embedded in glass, such as is also used to 'chip' dogs and cats so they are individually identifiable. We combine the transponder with a transponder reader at the nest box opening. Whenever one of the parent birds enters to feed the nestlings, the identity of its chip and the time are recorded. This technology works extremely well, and the data obtained has already been used in a large number of publications.

One of the problems, however, is that the available transponders are designed for domestic pets and not for birds. They are therefore relatively large (1.1 centimetres) and heavy (0.11 grams). Until recently, we glued the transponder onto a colour ring which we attached to the bird's leg. The ring plus transponder is 1.5 centimetres long and weighs 0.40 grams. That is quite big for a great tit weighing 20 grams or a pied flycatcher weighing 12 grams.

Recently, however, a transponder has been developed specifically for bird research. This smaller and lighter transponder is inserted directly into a plastic ring that can easily be attached to the bird. The whole thing weighs only 0.10 grams (four times lighter) and is only 0.8 centimetres long (half as long). This greatly reduces the level of distress for the bird, even though the same data can be collected. In 2014, all 132 transponder readers operated by the Netherlands Institute of Ecology were adapted and new, smaller transponders were purchased. These transponders and readers were used entirely satisfactorily in the field in the spring of 2014, with the discomfort for the birds being reduced. The switch to the new transponders is therefore in line with the objectives of the Three R's Fund.

Careful selection of animal species

Careful decisions are taken at the three institutes as to whether laboratory animals are in fact necessary to 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.

Animal Experiments Openness Code

The Academy strives for greater openness regarding animal experiments. Together with the Association of Universities in the Netherlands (VSNU) and the Netherlands Federation of University Medical Centres (NFU), it has therefore been actively involved in drawing up an Animal Experiments Openness Code [*Code Openheid Dierproeven*] (see also www.knaw.nl/nl/actueel/publicaties/code-openheid-dierproeven). Institutions that have signed up to the Code must account publicly for their

use of laboratory animals. 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 animal experiments involves various dilemmas. Being too open can put the Academy's 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.

Government Information (Public Access) Act

In accordance with its policy, the Academy is as open as possible in answering questions reaching it in the framework of the Government Information (Public Access) Act [*Wet openbaarheid van bestuur, Wob*]. This concerns both questions put to the Academy itself and questions regarding which it has presented its opinion as an interested party. The latter category includes questions directed to the Dutch Food and Consumer Product Safety Authority (NVWA) but also those put to the Central Committee for Animal Experiments under the new Animal Experiments Act [*Wet op de dierproeven, Wod*]. More information about this new legislation is given in Section 3, '*Supervision and monitoring*'.

English-language course in laboratory animal science

Since 2009, the Academy has organised an officially recognised annual course in laboratory animal science. In 2013 and 2014, numerous researchers at the Academy's institutes took the course,² as well as researchers from other organisations. Course participants visit the Academy's various laboratory animal facilities, with the focus being on the most commonly used species, such as mice and rats, but also on research involving non-human primates, fish, and animals from the wild or in their biotope. After successfully completing the course, participants are authorised to work with laboratory animals and to carry out animal experiments. They gain experience during training sessions given by colleagues from within the institutes or elsewhere and by attending courses or customised training.

² The course is basically intended for 24 participants. Some people take only the components on Dutch law and ethics because they have already been trained abroad.

2. THE THREE INSTITUTES AND THEIR RESEARCH

This section outlines the research using animals at the three Academy institutes that carry out animal experiments and house laboratory 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 from a fertilised ovum into an organism. One important research question is how organs come into being during embryonic development and grow to become functional components of an adult organism. The Hubrecht Institute also investigates what happens when organs are damaged, for example the heart or the pancreas. The institute uses vertebrates (mice, rats, zebrafish, frogs) and invertebrates (insects and nematodes) to gain fundamental knowledge that helps to understand genetic defects, as well as diseases such as cancer, diabetes, and heart failure. Amongst other things, the research provides leads for developing new treatment methods. The researchers are increasingly focusing on adult and/or embryonic stem cells. This is because these have many features in common with the rapidly dividing cells in tumours. Stem cells can also develop into specific tissues, for example for the heart, brain, intestines, and pancreas. Many doctors therefore believe that stem cells have great potential for use in treatment, but before stem cell therapy can be applied they 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. Researchers attempt to cure diabetic mice by isolating pancreatic stem cells, multiplying them in a test tube, and then injecting them back into the mouse. Mice are also used in efforts to determine the origin of blood stem cells. Researchers have also recently begun using mice to study heart failure. This involves investigating factors that have a positive or specifically negative effect on recovery after heart failure (see the box '*Eva van Rooij on researching heart disease*').

Rats are used to make 'genetic knock-outs': the animals lack the function of a certain gene and can consequently act as a model for humans with a defect in the same gene. By using zebrafish – and particularly their embryos – the biologists can study the development of the heart, bones, blood, and blood vessels. Other research questions include: How do diseases originate that affect the development of these organs? How can those diseases be prevented or cured? Researchers also use frogs' eggs to produce extracts with which they can study the mechanism underlying DNA repair.

The Hubrecht Institute is also known for the development of organoids. These are mini-organs, for example mini-intestines, that are made from stem cells. They replace some of the research using laboratory animals.

Eva van Rooij on researching heart disease

‘The heart is the body’s engine. It pumps the blood round to supply all the other organs with oxygen and nutrients. The heart of an adult human consists of approximately five billion heart muscle cells, which are together responsible for the pumping power of the heart. When someone has a heart attack (a ‘cardiac infarction’), a blood vessel supplying blood to the heart becomes clogged. Part of the heart then dies due to a lack of oxygen or nutrients. And because the heart cannot repair the damage itself, the infarction causes permanent damage and a reduction in cardiac function. That reduction can ultimately lead to heart failure, which causes many deaths every year.

Despite the lasting damage, the heart has various mechanisms that are activated during an infarction to protect the heart. Unfortunately, activation of those mechanisms is not effective enough to repair the damage caused by an infarction. Our research focuses on stimulating those protective mechanisms so that the heart’s function endures less damage.

We use mouse models in which we can study which genes are important as regards the damage caused to the heart after an infarction. By then overly-activating those genes in the heart or removing them from it, we can determine what their direct effect is on the amount of damage to the heart after an infarction. Our studies allow us to contribute to the development of new medication for patients who have had a cardiac infarction, in the hope that the damage can be limited and that they will not eventually develop heart failure.’

Netherlands Institute for Neuroscience

The Netherlands Institute for Neuroscience carries out basic research on how the brain functions, both in healthy people and in those who have a brain disorder or brain damage. Knowledge about brain diseases is becoming increasingly important because diseases of the nervous system form a very large group of debilitating disorders. That means not only Alzheimer’s or Parkinson’s diseases but also psychiatric illnesses, damage to the spinal cord, and strokes.

Laboratory animals play an important role in our research into the functioning of the brain and the origin of neurological disorders. Research can also be carried out using isolated tissues, for example, but to effectively map out complex processes that occur in living brains it is necessary to have a functioning biological system.

For brain research, we make use of zebrafish, mice, rats, and rhesus monkeys. Our basic principle is that the animals should be able to display their natural behaviour to the maximum extent. They are preferably housed in groups, and we make use of material that stimulates their natural behaviour. We refer to this as ‘environmental enrichment’. For some mice, we recently began using cages with additional enrichment, thus promoting social interaction and stimulating the animals to search actively for their food and water (see the box ‘*Environmental enrichment for mice*’ in Section 1).

Researchers at the Netherlands Institute for Neuroscience investigate cognitive functions, including consciousness, learning, movement and social interaction, both under normal conditions and in the presence of disease. They also study how nervous tissue recovers after damage (for example a spinal cord lesion) and how the light that enters the eye is converted into signals that go to the brain.

The institute uses rhesus monkeys in research on thought and concentration processes, indicators, for example, for Alzheimer's disease and ADHD. These animals are also being used to develop a prosthesis for the blind, in which camera images are presented directly to the visual cerebral cortex by means of electrical stimulation. The monkeys are housed in state-of-the-art accommodation, with environmental enrichment. They are kept in pairs in large cages. All the animals used have been born and reared in captivity; they have therefore not been captured in the wild. See also the webpages about '*Monkeys at the Institute for Neuroscience*': www2.nin.knaw.nl/onderzoek_met_resusapen.

Special mention should be made of research on deep brain stimulation. Researchers are constantly working to refine this technology, in which a pacemaker is used to reduce or boost activity in a particular area of the brain. The technology is already being used in Parkinson's disease sufferers, but the researchers are also trying to identify specific areas of the brain that play a role in the origin and persistence of obsessional neuroses and depression.

Maarten Kole on multiple sclerosis

'In people with multiple sclerosis (MS), there is something wrong with the myelin, the fatty material that insulates the axons and separates them from one another. MS sufferers undergo phases of spontaneous myelin loss in multiple regions of the brain. Our research group is trying to understand whether nerve impulses change if there is insufficient myelin. We are also interested in how cells wrap the right amount of myelin around the axons. And we hope to explain why more than half of MS sufferers develop memory problems.

We use mice in our research. We give them food containing a toxin that causes the brain cells that produce myelin to disappear. We have discovered that the nerve cells of our "MS mice" first become more active. That was surprising. It happens mainly at the beginning when the myelin is disappearing.

We also saw that the myelin-forming cells return when the mice are given normal food again. After a few weeks, there is hardly any difference between the brains of the MS mice that are again receiving normal food and those of animals that have not been treated. We are now investigating how that reversibility actually works. Imagine what that would mean if we could achieve the same effect in humans! We are also using the knowledge we have gained to predict the effects of certain drugs and to test them.'

Netherlands Institute of Ecology (NIOO)

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. Since 1955, the institute has been monitoring the nest boxes of great tits, blue tits, and pied flycatchers. Researchers count the eggs, ring the nestlings, and read the rings worn by the parent birds. Using this information, they investigate a wide range of topics, including the effects of climate change on nature, ageing in wild birds, and how male and female great tits share rearing their young.

In some cases, the birds are equipped with a tiny transmitter. In the case of great tits, for example, the researchers can tell whether the birds sleep more restlessly if their roosting site is illuminated by artificial light. This is one of the ways the NIOO is investigating the effects night-time illumination has on nature. A number of geese have also been equipped with a GPS data logger so that the researchers can see where they are currently located (see the box '*Bart Nolet on the advantages and disadvantages of migration*').

The Netherlands Institute of Ecology has a number of special aviaries where two family 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 the wild.

Bart Nolet on the advantages and disadvantages of migration

'Ever since Dutch sailors discovered back in the sixteenth century that barnacle geese spend the summer in the far north, people have wondered why they go so far north to breed. One hypothesis is that the young goslings need green, protein-rich grass in order to grow. However, the adult birds also need that kind of grass in order to lay their eggs. That poses a problem because the protein content of the grass falls in the course of the growing season. By equipping individuals with satellite transmitters or GPS loggers, we were able to demonstrate that the parent birds follow a kind of 'green wave' during the spring migration: they take advantage of the successive protein peaks at different latitudes over the course of time. More specifically, they catch up with the green wave. In this way, the parent birds are ready to lay their eggs in the northern breeding grounds and the chicks can benefit from the local protein peak.

Barnacle geese now also breed in the Netherlands, giving us a unique opportunity to carry out comparative research on the advantages and disadvantages for the birds of migrating to the north. Working with researchers from the University of Groningen, we have shown that the northern barnacle geese invest less in their defence system than those that breed in the Netherlands. That is probably because there are fewer pathogens up in the north.

That is offset by higher energy expenditure by the birds that breed in the north. We measure their metabolism in the field to test our idea that the birds in the far north have a faster pace of life. We also use advanced GPS loggers – which record behaviour as well as location – to quantify how much more than the 'sedentary' (non-migrating) birds the migratory birds actually fly (which requires a great deal of energy). After all, the sedentary birds also fly back and forth between where they feed and where they roost. We are doing this type of research so as to ultimately be able to predict whether and how the birds can adapt to the rapid changes taking place in their environment.'

The Dutch Centre for Avian Migration and Demography (housed at the Netherlands Institute of Ecology)

The Dutch Centre for Avian Migration and Demography is the primary centre of expertise in its field in the Netherlands. It is a partnership between the Netherlands Institute of Ecology (NIOO-KNAW) and the Ringers Association [*Ringersvereniging*], and administers the day-to-day work of catching and ringing birds for scientific research.

Although new technology such as transmitters and loggers is becoming more sophisticated and can generate a wealth of information about bird movements, ringing remains by far the most frequently used method for tracking birds. It is relatively simple and inexpensive. The degree of distress is limited mainly to the moment of capture and the brief time that the bird is held so that it can be measured, weighed, and ringed.

Each year, voluntary and professional bird ringers ring some 300,000 wild birds in the Netherlands with a metal ring so as to record their movements and lifespan. The data on the ringed birds and the reports that they generate form the input for research.

That research currently focuses on collecting demographic data about birds: standardised catching and ringing of large numbers of birds provides information about their reproduction and survival. That information plays an important role in studying the effects of climate change, but also in identifying the factors underlying changes in bird numbers.

There are currently 530 active ringers in the Netherlands, most of them volunteers. There has been a steady rise in that number in recent years, and this is expected to continue for a while, ultimately stabilising at about 600. Without the efforts of the volunteers, for whom catching and ringing birds is often more of a life's work than just a hobby, the research would not be possible.

Catching and ringing wild birds is not covered by the provisions of the Animal Experiments Act, but ringers do require an exemption under the Dutch Flora and Fauna Act [*Flora- en faunawet*]. Thanks to a 'framework exception' the Centre for Avian Migration and Demography can mandate individuals to catch and ring birds.

Ringers are regularly deployed in research that does in fact involve action that constitutes an animal experiment. The fact that ringers have a high degree of expertise and can catch birds in all kinds of locations and during all seasons makes them sought after as collaborators. Almost a quarter of ringers are authorised to carry out animal experiments.

Animal experiments in which ringers are increasingly involved are blood sampling or collecting feathers for virological and genetic research; taking smears to determine infection with avian influenza; and attaching loggers and transmitters.

The Centre for Avian Migration and Demography is receiving more and more requests for collaboration from Dutch and foreign researchers. It is not always possible to comply with these requests, however, because the ringers in question do not hold the necessary authorisation. There is a pressing need for a tailor-made course enabling ringers and other field workers to learn both specific relevant skills and to acquire their authorisation. Such a course is currently under consideration.

3. SUPERVISION AND MONITORING

The Academy's Animal Experiments Committee (DEC) checks every research plan to determine whether the use of an animal is justified by the scientific and public interest of the proposed research. The Animal Experiments Act [*Wet op de dierproeven*] prohibits animal experiments if alternatives are available, if other – often 'lower-order' – animals can be used, or if the experiment can be carried out in a manner that causes less distress to the animals concerned. The Animal Experiments Committee 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 laboratory animal experts on staff. Their task is to monitor animal welfare, to inspect whether each animal experiment is performed as described in the research plan, and to check whether the conditions set by the Animal Experiments Committee are being complied with. They regularly visit the locations where the animals are housed and the experiments are carried out. The two experts also update the relevant records and make recommendations to the Animal Experiments Committee. They have a monthly consultation meeting with the Academy's Director of Research; it is he who is the legal permit-holder for animal experiments.

Animal Experiments Committee

The Academy's Animal Experiments Committee holds monthly meetings. Its members are experts on animal experiments, laboratory animals and their protection, alternatives, and ethics. The Committee consists of:

- an independent chairperson not employed by the Academy and not involved in the animal experiments;
- an independent expert in the field of ethics who is not involved in the animal experiments;
- an independent expert on laboratory animals 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 with regard to laboratory animals 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, but he/she is allowed to participate in the discussion.

The Academy's policy for the near future is for the whole of the DEC to consist of external independent members, thus pre-empting any suggestion that 'the fox is guarding the chickens'.

Procedure and exceptions

A lengthy procedure has already taken place before the Animal Experiments Committee considers 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 laboratory animal experts then discuss the plan with the researcher. The research plan is subsequently submitted to the Animal Experiments Committee.

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.³ 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 Animal Experiments 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 laboratory animal experts. If there is even the slightest doubt – for example regarding the risk of distress – then the research plan must be submitted to the Animal Experiments Committee.

³ The Academy's standing rules provide that certain experiments can be dealt with outside the regular meetings of the DEC. This concerns experiments involving only slight distress for the animals (with the exception of primates), experiments with moderate distress that are performed repeatedly according to a fixed protocol and that have previously been assigned a positive recommendation by the full DEC, or amendments to current research plans in which there is no change in the level of distress or in the research question. Such research plans and amendments can be assigned a recommendation by a subcommittee, with the laboratory animal experts as advisers. The membership of this subcommittee need not always be the same. Amendments requesting an extension of the period for which the original recommendation applies can also be dealt with by a subcommittee. These recommendations are sent to the DEC for information purposes and are discussed at a meeting of the full DEC.

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. They evaluate the expertise of the researchers and support staff, and how the animals are housed. In some cases they observe animal experiments as they are being carried out. In recent years, they have requested additional information about exemption requests and have inspected the records for particular types of animal experiments.

The new Animal Experiments Act (Wod)

The new Animal Experiments Act (Wod) came into force on 18 December 2014. This legislation has major consequences for the working methods of the Academy's Animal Experiments Committee (DEC), and for all other parties involved in such experiments. At the end of 2014 some aspects of the legislation and regulations were still unclear, but it is hoped that there will be clarified in the course of 2015.

In the months prior to the new legislation coming into force, the DEC received more research proposals and research amendments for review than usual, largely because many researchers were uncertain of the new Act's impact on the progress of their research.

4. FIGURES

4.1 Number of research plans

	Netherlands Institute for Neuroscience	Hubrecht Institute	Netherlands Institute of Ecology	Total
2009	68	41	12	121
2010	65	41	12	118
2011	96	65	10	171
2012	107	81	19	207
2013	71	62	14	147
2014	120	83	18	221

Number of research plans submitted by Article 9 officials at the various institutions.

4.2 Recommendations by the Animal Experiments Committee

	Positive recommendation immediately	Positive recommendation after revision of plan	Negative recommendation or withdrawal of research plan
2009	86%	11%	3%
2010	86%	9%	5%
2011	91%	7%	2%
2012	86%	13%	1%
2013	84%	13%	3%
2014	91%	9%	-

Recommendations by the Animal Experiments Committee to the permit holder regarding the research plans considered. The permit holder has indicated that the recommendations will be complied with.

4.3 Number of animal experiments

	2010	2011	2012	2013	2014
Mice	6,621	8,495	8,755	9,697	7,593
Rats	1,496	1,508	1,659	1,338	1,653
Fish	2,168	1,558	732	654	1,641
Birds	5,032	8,640	6,533	4,596	4,163
Non-human primates	2	7	4	7	-*
Rabbits	-	-	-	-	-
Amphibians	-	-	5	256	247
Total	15,319	20,208	17,688	16,548	15,297

*Annual number of animal experiments, by animal species. An animal experiment involves the use of a single animal for a single experiment. The same animal may be used in more than one experiment, in which case it is then counted twice. Only experiments completed in the relevant year of registration are shown. *In 2014, for example, no experiments using non-human primates were completed. No distinction is made between genetically modified animals and animals from the wild.*

4.4 Distress

	slight	slight/ moderate	moderate	moderate/ serious	serious	very serious	total
2009	4,227	6,135	2,084	1,849	-	-	14,295
2010	5,122	7,619	1,581	997	-	-	15,319
2011	8,226	8,555	2,403	1,024	-	-	20,208
2012	4,503	8,414	3,673	1,098	-	-	17,688
2013	6,506	5,540	3,432	1,070	-	-	16,548

	terminal under anaesthesia	slight	moderate	serious	total
2014	602	13,038	1,657	-	15,297

Up to and including 2013, registration involved six categories of distress (from slight to very serious). Since 2014, it has been categorised as terminal under complete anaesthesia, slight, moderate, or serious.

4.5 Inspections

	prior notice given	no prior notice given	total
2009	6	1	7
2010	1	3	4
2011	7	5	12
2012	4	5	9
2013	2	5	7
2014	4	3	7

Inspections are carried out by staff of the NVWA.

5. FREQUENTLY ASKED QUESTIONS

The Dutch Society for the Replacement of Animal Testing [*Stichting Proefdiervrij*] has drawn up a ‘Transparency Benchmark’ for research using animals. This is a kind of yardstick comprising a large number of questions. Some of those questions have already been answered above; others will be answered below.

1. What is the mission of the Academy’s institutes? Why are animal experiments necessary to pursue that mission? What role do laboratory animals play in achieving the Academy’s research priorities?

The Academy has sixteen institutes, three of which use laboratory animals. Those three are the Netherlands Institute for Neuroscience (Amsterdam), the Hubrecht Institute for Developmental Biology and Stem Cell Research (Utrecht), and the Netherlands Institute of Ecology (NIOO) (Wageningen). The mission of the various Academy institutes is not only to be among the world leaders in research but also to play a visible role in solving society’s problems concerning disease and health or in the area of climate and sustainability. In this research, laboratory animals play an important role as models for basic biological processes (for example the development of cancer cells or the regeneration of nerve cells after injury), but also, for example, in studying how birds respond to climate change. This kind of ecological research concerns the animals’ behaviour.

2. What is the Academy’s opinion regarding animal experiments? What research does the Academy not carry out as a matter of principle?

The Academy’s full opinion regarding this matter can be found on its website.⁴ Briefly, the Academy considers that animal experiments remain necessary in some research, albeit subject to strict conditions. The Academy is aware that such experiments are a matter of public debate, and it is receptive to that debate. The Academy considers that the legal frameworks set out in the Animals Experiments Act (Wod) constitute the minimum requirements for animal experiments and for laboratory animal housing. The basic principle is that no animal experiments should be carried out if there is an alternative to using them. In practice, this means that stringent demands are imposed within the research organisation as regards care and transparency. Further principles underpinning the Academy’s laboratory animals policy are the provision of objective information and maximum openness about animal experiments, with the intention of entering into public dialogue regarding this matter.

The Academy’s institutes do not carry out any contract research for companies, or any other commercially oriented research. Nor does any research take place in which the brains of non-human primates are damaged in order to then study processes of recovery. The latter type of research is prohibited at the Academy’s institutes.

⁴ www.knaw.nl/publieksstandpunt-dierproeven.pdf

3. How is research involving animals regulated? What is the nature of the research chain, where do the research assignments come from, and how is research funding arranged?

The great majority of research is funded research, with the aim being for basic research to make a demonstrable contribution to finding therapeutic solutions for serious medical conditions. The importance of this must at all times be weighed up against the disadvantages of animal experiments. Funding comes from the national research funding organisation, namely the Netherlands Organisation for Scientific Research (NWO), from charities that raise funds from the public (for example the Dutch Heart Foundation), or from the European Commission. The great majority of the Academy's research is publicly funded. The Academy's institutes do not perform any commercial, market-oriented research, or company-commissioned animal experiments.

4. What are the views of the Academy regarding the use of laboratory animals? On what assumptions and principles does it base its policy?

The current state of knowledge in biology and the biomedical sciences is to a large extent due to the use of laboratory animals. The Academy considers that animal experiments remain necessary for certain biological and biomedical research because alternatives that are of equivalent or superior value are so far unavailable. In some fields of research, alternatives are becoming available and the number of animal experiments is declining. The Academy is encouraging these developments. The research carried out by the Hubrecht Institute using organoids is a clear example of this.

5. How is the legislation complied with in practice?

The Academy's laboratory animal experts have the authority to stop a study immediately if it fails to comply with the legal requirements. The Academy considers this to be an important matter, and it also has plans to add more manpower, in particular to further tighten up supervision. A policy has also been adopted to the effect that the DEC should consist solely of external members, even though that is not a legal requirement. This allows the Academy to ensure that 'the fox is not guarding the chickens'.

6. How does the Academy deal with contract research requiring animal experiments?

The Academy's institutes do not carry out research of that kind.

7. Does the Academy make use of facilities belonging to other parties? In that case, how does it ensure that they also comply with the relevant requirements?

In general, the Academy does not make use of facilities belonging to other parties. Where that does occasionally take place, it is still the case that the Academy's DEC issues recommendations on all planned studies so that the Academy remains responsible for all research involving animals carried out by Academy researchers. There is no question of the Academy relaxing its own standards and monitoring role by assigning the responsibility elsewhere.

8. When programming research, does the Academy take account of ethics and of laboratory animals?

Yes, as is shown, for example, by the fact that the Academy's institutes do not carry out certain types of research. That applies to commercial research involving laboratory animals but also to research using lesions (see also question 2). The Academy has also published an opinion on research involving non-human primates (rhesus monkeys): although it is of great scientific importance, that research should not be expanded.

9. Does the Academy take account of social trends regarding the use of laboratory animals?

Yes, it goes without saying that the Academy takes account of changes in the way society views the use of laboratory animals. Opinions on the acceptability of animal experiments vary greatly, however. Patient organisations, for example, want research using laboratory animals to continue as long as there are no alternatives. Other people say that there are already alternatives, and that laboratory animals do not provide a good model. What is also problematical is that discussion sometimes wrongly assumes that researchers continue carrying out animal experiments against their better judgement.

10. Does the Academy engage in discussion with the stakeholders? If so, with which parties? And what about in the longer term?

Yes, and that is necessary and important. There are various types of stakeholders: scientists, politicians, the ministries, civil society groupings (patients, opponents). The Academy also issues recommendations regarding laboratory animals and animal experiments at the request of members of government. One example is the advisory memorandum from 2014 on whether it is useful or necessary to make use of non-human primates in research [*Gebruik van niet-humane primaten als proefdier – nut en noodzaak?*] (see also www.knaw.nl/nl/actueel/nieuws/aanbevelingen-voor-verbetering-van-wetenschappelijk-onderzoek-met-apen).

The institutes themselves are also active in this respect. The Hubrecht Institute, for example, has an outreach programme, the NIOO organises open days, and the Netherlands Institute for Neuroscience has special webpages on research using rhesus monkeys (www2.nin.knaw.nl/onderzoek_met_resusapen).

11. What role do funding bodies/clients play in discussions with the stakeholders?

They do not play any role. The Academy determines its position independently.

12. Do the stakeholders have an influence on the Academy's policy? On what matters does the Academy report to stakeholders, and how does it do so?

The stakeholders always have an influence because they are a reflection of the public debate. The Academy regularly talks to representatives from the field (both supporters and opponents of animal experiments). It does so by organising conferences and debates.

13. With what dilemmas does the Academy find itself struggling? How does it tackle them? And how do you foresee the future?

Research using monkeys gives rise to dilemmas, for example. It is world-class work and more experiments should therefore be carried out. We will not be doing this,

however, because the Academy takes account of public opinion when considering such research.

Another dilemma arises from the desire to reduce the number of laboratory animals, while at the same time the Academy's researchers have been increasingly successful in competing for grants that bring new scientific fields within reach. The volume of research consequently increases, meaning that researchers sometimes end up using more animals instead of fewer. A similar dilemma arises from the availability of completely new technology that makes it possible to equip more species of birds with transmitters and track them for some time.

14. How does the Academy respond if the DEC alerts it to dilemmas?

In the first instance, there is a discussion with the permit-holder for animal experiments, but where relevant, external advice is sought, for example from other DEC's.

15. How does the Academy see the use of laboratory animals developing in future? And is the Academy making efforts to reduce the number of laboratory animals used?

The Academy believes that in the longer term there will certainly be a reduction in the number of animal experiments, but it is difficult to say how quickly that will happen. Scientific research is dynamic: new fields are continually developing that our previous lack of knowledge prevented us from undertaking before. It is also relevant that research that was not previously classified as animal experimentation will now be classified as such. One example is the equipping of birds with an external transmitter.

16. How does the Academy monitor of the use of laboratory animals internally, and how are the rules enforced? (For example, in terms of housing, care, and treatment and the associated enforcement; anaesthesia and euthanasia and the associated compliance; experimentation and the associated enforcement.)

This is a crucial point. The Academy complies with all the legal requirements, and indeed does more. Accommodation is properly arranged, and acts as an example for some non-Academy institutions. The Academy's institutes are also regularly visited – with and without prior notice being given – by inspectors from the Dutch Food and Consumer Product Safety Authority (NVWA). If there is anything wrong, the permit-holder receives a warning and if several warnings are issued an official report may be made. Where monitoring is concerned, the Academy plans to add additional staff so that not only the DEC but also the new Animal Welfare Body can perform its duties to the optimal extent.

17. What is the Academy doing to avoid the use of laboratory animals? Namely as regards research methods and the formulation of research questions?

The Academy has set up its own 'Three R's Fund' for its institutes. This provides an annual sum of EUR 100,000 which researchers can call on for support in finding alternative methods, or for reducing, replacing, or refining animal experiments. In reality, it is proving difficult to reduce the absolute number of laboratory animals used in experiments, in part because the Academy's researchers have been so successful that the institutes are growing in size (see also question 13). There are also some problems with the Academy's own Three R's Fund. We must see that the funds are not invested mainly in refinement – after all, replacement and reduction are also essential.

18. What are the most important research programmes (in outline/clusters) in which laboratory animals are used?

Virtually the entire research programme of the Netherlands Institute for Neuroscience and the Hubrecht Institute involves animal experiments. That is otherwise at the NIOO, which also carries out a lot research that does not require laboratory animals to be used.

19. Does the Academy wish to add anything?

The Academy is very well aware of the Dutch population's views on animal experiments and laboratory animals, and how those views are changing. At the same time, the Academy is also aware that opinions diverge considerably and are sometimes mutually exclusive, making it difficult for opponents and supporters to treat one another respectfully. Against that background, the Academy recently decided to alter the requirements for the chair of the DEC. The new chair is no longer a scientist experienced in animal experimentation but a vet-lawyer with a considerable background in public debate.

6. BACKGROUND INFORMATION

Terms

This annual report utilises terms and concepts as explained below:

- **Article 9 official:** A person who directs the performance of the research.
- **Article 12 official:** A person who performs the animal experiment.
- **Article 14 official:** A person who ensures the welfare of the animals.
- **Research using animals:** This includes the experiment itself, the breeding of animals intended for use in research, and the maintenance of a breeding strain of laboratory animals.
- **Animal experiment:** The use of a single animal for a single experiment.
- **Non-human primates:** Collective name for rhesus monkeys, cynomolgus monkeys, and marmosets. The Netherlands Institute for Neuroscience uses only rhesus monkeys.
- **Use of laboratory animals:** The use of one or more animals for one or more experiments. This includes the housing, care, treatment, anaesthesia, and euthanasia of these animals.

Abbreviations

DEC: Animal Experiments Committee

AWB: Animal Welfare Body

Academy (KNAW): Royal Netherlands Academy of Arts and Sciences

NFU: Netherlands Federation of University Medical Centres

NIOO-KNAW: Netherlands Institute of Ecology

NVWA: Dutch Food and Consumer Product Safety Authority

NWO: Netherlands Organisation for Scientific Research

VSNU: Association of Universities in the Netherlands

Wob: Government Information (Public Access) Act

Wod: Experiments on Animals Act

Webpages in the annual report

- www.knaw.nl/nl/thematisch/ethiek/dierproeven/overzicht: Special website with more information about animal experiments and the Academy.
- www.knaw.nl/publieksstandpunt-dierproeven.pdf: In 2010, the Academy published its opinion on research involving laboratory animals and its importance.
- www.knaw.nl/nl/actueel/publicaties/code-openheid-dierproeven: The Animal Experiments Openness Code
- www.knaw.nl/nl/instituten/hubrecht-instituut: Hubrecht Institute for Developmental Biology and Stem Cell Research. See also: www.hubrecht.eu.

- www.knaw.nl/nl/instituten/nederlands-herseninstituut: Netherlands Institute for Neuroscience. See also: www.herseninstituut.knaw.nl.
- www2.nin.knaw.nl/onderzoek_met_resusapen: Webpages about the use of monkeys at the Netherlands Institute for Neuroscience
- <http://www.knaw.nl/nl/instituten/nioo>: Netherlands Institute of Ecology. See also: www.nioo.knaw.nl.
- www.vogeltrekstation.nl: Dutch Centre for Avian Migration and Demography (housed at the Netherlands Institute of Ecology).
- www.knaw.nl/nl/actueel/nieuws/aanbevelingen-voor-verbetering-van-wetenschappelijk-onderzoek-met-apen: The Academy's advisory memorandum from 2014 on whether it is useful or necessary to make use of non-human primates in research [*Gebruik van niet-humane primaten als proefdier – nut en noodzaak?*].