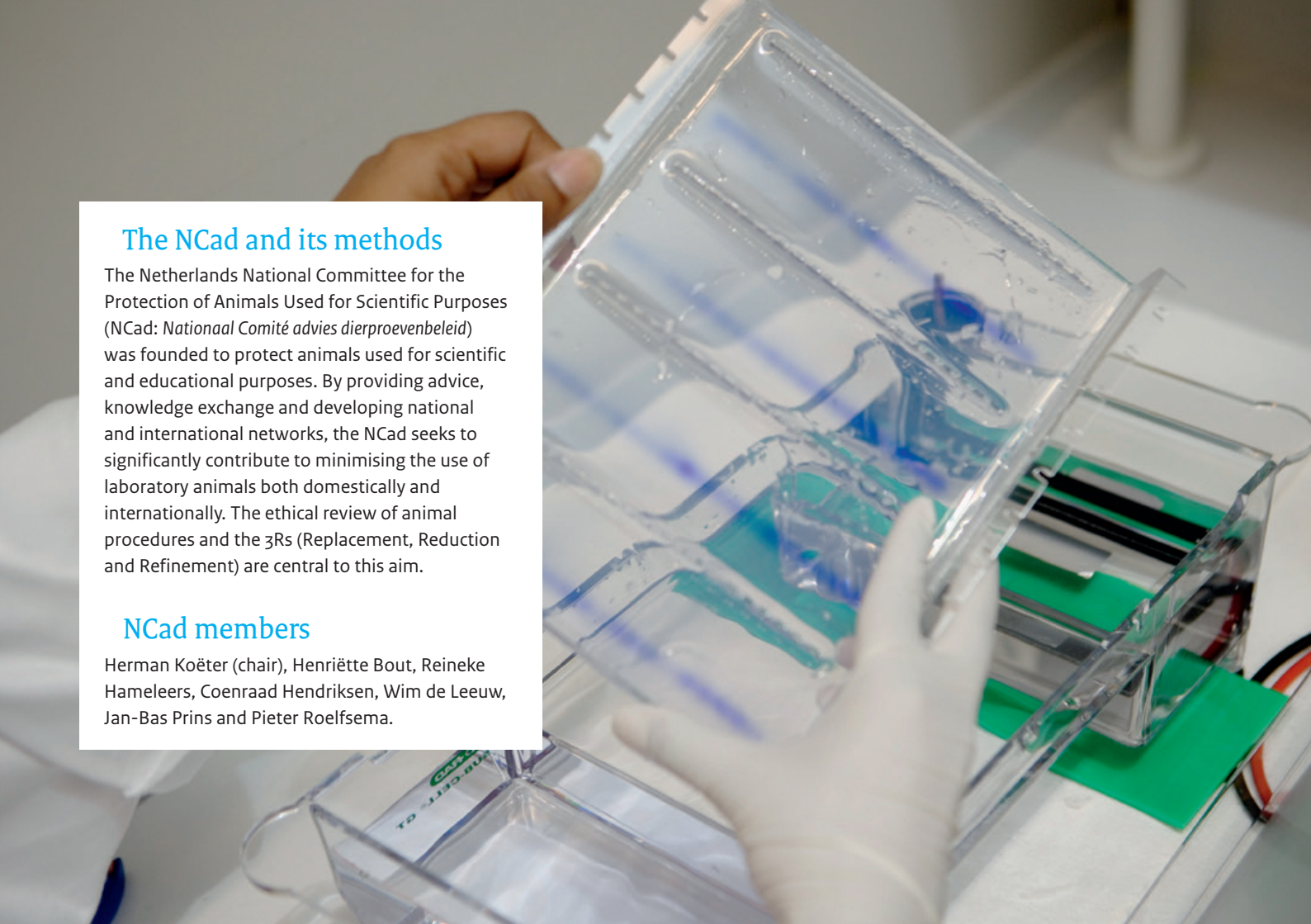
Netherlands National Committee
for the protection of animals
used for scientific purposes

Advisory report on genetically modified animals

'Died or killed before use in breeding programmes or animal procedures' Part 2: Quality criteria

Advisory report by the Netherlands National Committee for the Protection of Animals used for scientific puposes (NCad) at the request of the Minister of Agriculture, Nature and Food Quality.



The NCad and its methods

The Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad: *Nationaal Comité advies dierproevenbeleid*) was founded to protect animals used for scientific and educational purposes. By providing advice, knowledge exchange and developing national and international networks, the NCad seeks to significantly contribute to minimising the use of laboratory animals both domestically and internationally. The ethical review of animal procedures and the 3Rs (Replacement, Reduction and Refinement) are central to this aim.

NCad members

Herman Koëter (chair), Henriëtte Bout, Reineke Hameleers, Coenraad Hendriksen, Wim de Leeuw, Jan-Bas Prins and Pieter Roelfsema.

Summary

In 2015, the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) issued an advisory report (Part 1) regarding the reduction of the number of genetically modified (GM) animals in the category of "Died or killed in stock", with a particular focus on fish and mice.
The report stated that successive technological developments are taking place at an ever-increasing rate and that "genome editing" techniques could, to a large extent, replace the traditional transgenic methods.
In order to improve the quality of the generation and breeding of genetically modified animals in the Netherlands and make all parties concerned aware of the specific, defining moments at which it is possible to reduce the number of animals killed before they are used in breeding or animal procedures, the NCad has issued a supplementary advisory report (Part II), which sets out specific quality and efficiency criteria for bringing about a reduction in the aforementioned numbers. These criteria also serve as a guide for taking properly justified decisions. Hence, this advisory report does not address the fundamental question of whether GM animals should be created and bred in the first place.

In its discussions, the NCad has extensively considered the question of whether or not it is expedient to issue the advisory report. This is due to the dilemma that, on the one hand, gene editing techniques can be used to create a genetically altered animal that is tailor-made for a given experiment while reducing the number of animals required for animal experiments. However, on the other hand, genome editing techniques likewise offer new "tailor-made" opportunities for studying health issues. This can lead to an increase in the number of breeding lines and to a corresponding increase in the absolute number of animals killed before use in breeding programmes or animal procedures. After ample consideration, the NCad nevertheless decided to issue this advisory report because it contributes to the intended purpose and addresses the dilemma described above.

The NCad provides the following five recommendations to the Minister:

- Advise the Central Authority for Scientific Procedures on Animals (CCD) to follow the Codes of Practice (CoP) as included in Appendices 1 and 2 as the basic principles for granting project permits. The quality and efficiency criteria listed in Appendix 1 will help optimise the entire chain for the modification and breeding of genetically modified animals.

- Involve what are referred to as "centres of excellence for genetic engineering", also known as Gene Technology Platforms (GTPs), in both the planning and generation of GM lines. A GTP possesses the knowledge required for providing advice on the best way to create a certain GM animal model, as well as the competence and infrastructure for subsequently generating the GM animal model as efficiently as possible. The researcher must belong to or at least have access to such a GTP.

- Al the GTPs provide an overview of the available GM lines. This requires close collaboration and openness between the different research institutes. The initiative for this should be taken by the GTPs in collaboration with the animal testing facilities.

- Assign the task of coordinating the breeding process to the animal testing facility and ensure that access to the right expertise and facilities is also provided for in this task. The cryopreservation and rederivation strategy is another integral part of the breeding coordination. Decisions regarding the breeding strategy to be followed and its implementation should be made in close consultation with the responsible researcher and the Animal Welfare Body (IvD).

- Initiate a social dialogue – similar to the Nanopodium platform – regarding the possible applications of "gene editing". This is important since genetic modification is a socially sensitive issue, for example because it is also possible to make genetic modifications in animal species other than mice.

The appendices include two CoPs drawn up by two expert working groups based on the latest insights. The NCad is of the opinion that the use of the criteria and quality standards stated therein will contribute to optimising the entire chain for the modification and breeding of genetically modified animals.

Table of contents

1. Introduction

At the end of 2015, in response to a question from the State Secretary for Economic Affairs, the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) issued its first advisory report (Part 1)[1] regarding the reduction of the number of genetically modified (GM) animals in the category "Died or killed in stock", with a focus on fish and mice.

Part I of the report refers to the successive technological developments that are taking place at an ever-increasing rate, and states that "genome editing" techniques could, to a large extent, replace the traditional transgenic methods. It was expected that this would make it possible to generate GM animals in a more efficient manner. However, in practice traditional and new transgenic methods were used side by side because gene-editing methods are not necessarily the best option for the creation of any new GM animal.

Based on the "no, unless" principle, it should be determined for every new GM animal to be created whether the research objective can be achieved using a non-animal method. A licence from the Central Authority for Scientific Procedures on Animals (CCD), partly based on an ethical evaluation by an Animal Ethics Committee, constitutes the starting point of Part 2 of the underlying advisory report. This advisory report therefore does not address the fundamental question of whether GM animals should be created and bred in the first place.

[1] **https://www.ncadierproevenbeleid.nl/documenten/rapport/2015/11/26/advise-stock-animals**

The advisory report sets out quality and efficiency criteria that should contribute to reducing the number of GM animals killed before use in a breeding programme or animal procedure, as stated in the new registration system.[2]

Animals used for animal procedures are usually also bred for that purpose. The first stage in the GM animal process entails introducing a genetic modification into the parent animals (founders) before a sufficient number of animals with the appropriate genetic modification can be generated by means of a breeding programme. These animals are subsequently used in an animal procedure. Many animals born under these breeding programmes do not have the correct genetic makeup or are unsuitable for the intended animal procedure for another reason. The NVWA's 2016 registration data show that 232,472 GM mice were killed before use in a breeding programme or animal procedure compared to 78,209 registered animal procedures with GM animals.[3] In view of the principle that an animal has an intrinsic value, this is an undesirable situation. The number of GM mice killed before use in a breeding programme or animal

[2] NVWA registration of laboratory animals and animal procedures – until registration year 2013, dead or sacrificed animals were recorded under the following categories: "died or killed in stock before the start of the procedure", "died or killed during or in the context of the procedure", "died or killed following the procedure"; with effect from the 2014 registration year, the following categories were used: "died or killed before being used in breeding programmes or animal procedures", "after being used in breeding programmes", "during or in the context the procedure", "after use in the procedure". Sources: Explanatory notes to the registration of laboratory animals and animal procedures 2014.

[3] *Zo doende 2016* – Annual Review of Animal Procedures and Laboratory Animals published by the Netherlands Food and Consumer Product Safety Authority, Utrecht 2018.

procedure is around 70% of the total number of animals registered in this category. Therefore, if it is necessary to create and breed GM animals, it is important to ensure that this is done in a careful manner with a minimum loss of animals.

The NCad set up two working groups that surveyed the entire GM animal generation and breeding supply chain to identify the underlying reasons for the high number of GM animals killed before use in breeding programmes or animal procedures. Based on this survey, efficiency and quality criteria were formulated for generating and breeding GM animals with the aim of reducing the number of animals killed before use in the breeding programme or animal procedures.

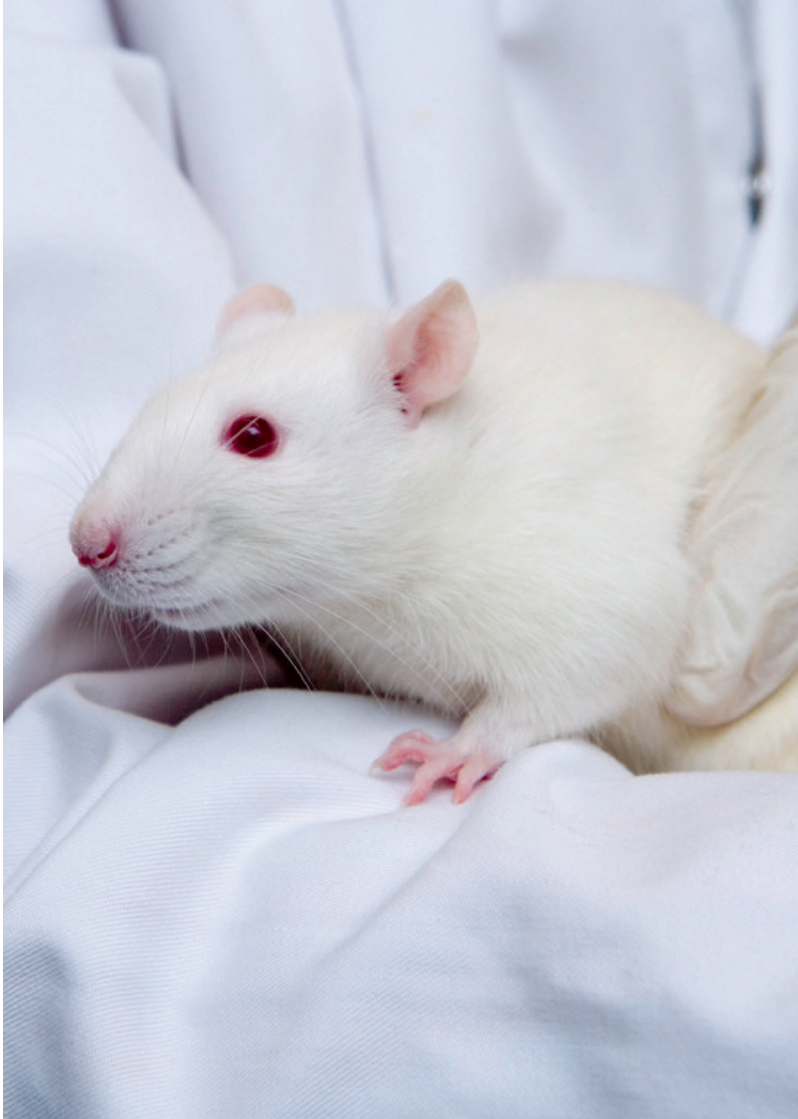
In addition to enhancing the quality of the process of generating and breeding genetically modified animals in the Netherlands, the criteria should make all parties involved aware of the defining moments at which a reduction in the number of animals killed before use in breeding programmes or animal procedures can be achieved. These criteria serve as a guideline for making well-motivated(L) decisions on these points.
As long as the use of GM animals and hence the generating and breeding of these animals is considered necessary and acceptable in the licensing procedure, the NCad considers it essential for the purpose of Refining and Reducing animal procedures to adopt the quality and efficiency criteria described in this advisory report as basic principles for this practice.

2. Request for opinion

In her request for an opinion of 31 March 2015, the State Secretary for Economic Affairs asked the NCad to comprehensively define the efficiency and quality criteria for the generation of genetically modified animals within so called “centres of excellence”. Such criteria should enhance the quality of breeding genetically modified animals in the Netherlands and make breeders aware of potential reduction in the total number of animals used for this purpose. It would be advisable to initially focus these criteria on the generation of genetically modified mice and fish (using both traditional and innovative methods) under own management. The State Secretary requested the NCad to consider the 2014 recommendation from the Regular Consultation Platform on Animal Procedures and Alternatives (RODA) in this process, as well as the results of the international workshop “Bred but not used” and the 2011 recommendation from the then Central Authority for Scientific Procedures (CCD) relating to the centralised breeding of transgenic animals.

Building on Part 1 of the advisory report, “Genetically modified animals killed in stock”, issued in October 2015, the NCad has now issued its second advisory report.

Part 2 elaborates on the recommendation formulated in the first advisory report to have additional criteria and quality standards drawn up by research groups or facilities.

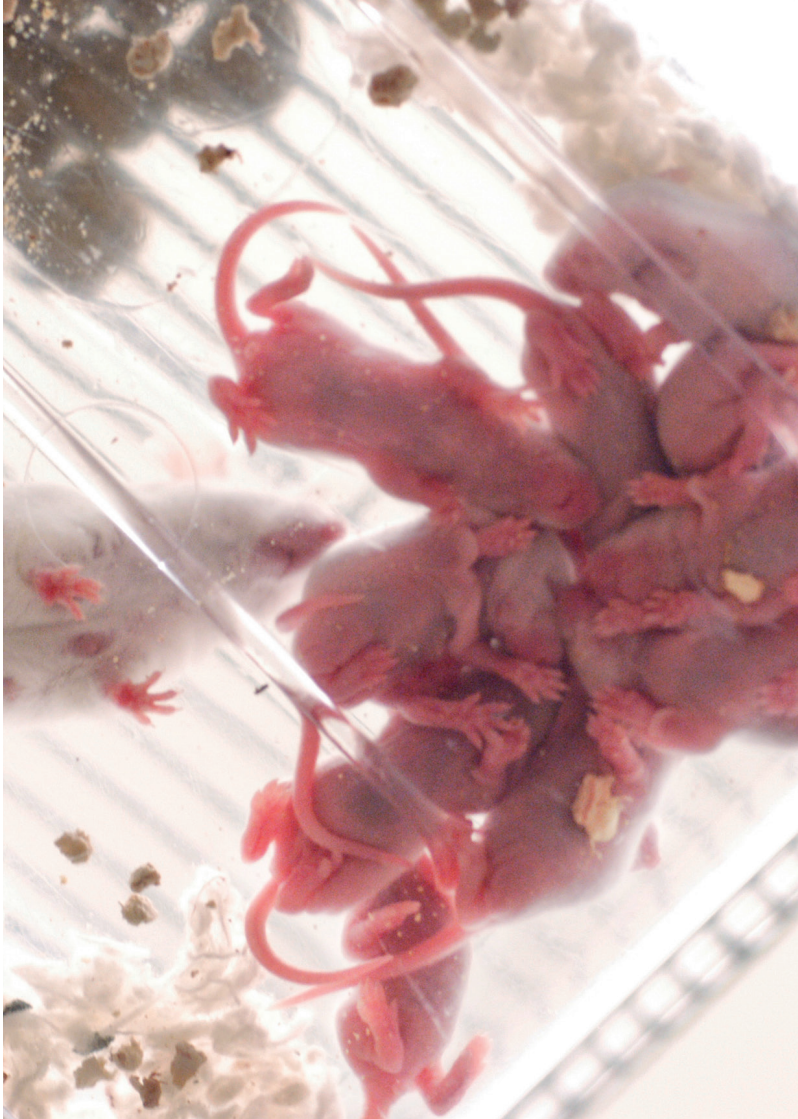


3. Prior considerations

During its discussions, the NCad extensively considered whether or not it is expedient to issue this advisory report. This is due to the dilemma that, on the one hand, gene editing techniques can be used to create a genetically altered animal that is tailor-made for a given experiment while ((reducing the number of animals required in the process). However, on the other hand, genome editing techniques likewise offer new "tailor-made" opportunities for studying health issues. This can lead to an increase in the number of breeding lines and to a corresponding increase in the absolute number of animals killed before use in breeding programmes or animal procedures. This development is incompatible with the policy-related aim to reduce the number of animals killed in stock in absolute terms.

After ample consideration, the NCad nevertheless decided to issue this advisory report because it contributes to the intended purpose. After all, it is expected that the relative number of animals killed in stock will decline upon the implementation of the Code of Practice accompanying this advisory report.

Moreover, the NCad has formulated two recommendations that endeavour to address the above dilemma.



4. Advisory report

4.1 Advise the Central Authority for Scientific Procedures on Animals (CCD) to follow the Codes of Practice (CoP) as included in Appendices 1 and 2 as the basic principles for granting project licences.

The NCad advises the CCD to apply the criteria and quality standards included in Appendices 1 and 2 and to adopt the preconditions and frameworks set out therein.

The generic application of the quality and efficiency criteria stated in Appendix 1 will contribute to optimising all aspects[4] of generating GM animals and breeding GM lines.

4.2 Involve "centres of excellence for genetic engineering" in both planning and generating of GM lines.

There are several centres of excellence for genetic engineering in the Netherlands. These "genome technology platforms" (GTPs)[5] have the knowledge required for providing advice on the best way to create a certain GM animal model, and have the competence and infrastructure to subsequently generate the GM animal model as efficiently as possible.

The researcher should belong to or at least have access to a GTP.

4.3 Letthe Gene Technology Platforms (GTPs) provide an overview of the available GM lines.

The provision of an overview of the available GM lines requires close collaboration and openness between the various research institutes. The initiative in this area should be taken by the GTPs in collaboration with the animal testing facilities.

The GTPs and the researcher should ensure that the newly created genetic modifications and lines are also reported to the international databases. The NCad is aware of the limiting factors, such as the need to protect intellectual property and privacy concerns.

4.4 Assign the task of coordinating the breeding programme to the animal testing facility and ensure that the cryopreservation and rederivation strategy form an integral part of this task.

The NCad recommends assigning coordination of the breeding programme to the animal testing facility. This task should include the responsibility for and the provision of access to the appropriate expertise and facilities. Decisions regarding the breeding strategy to be followed(L) and its implementation should be made in close

[4] This should include generating genetically modified animals (mice); acquiring genetically modified animals; cryopreservation; rederivation; breeding GM lines; and cross-breeding and backcrossing with other lines and strains.

[5] A GTP is a core facility, either public or private, which possesses the knowledge and expertise to supervise and perform the entire process from design to the creation of a GM animal model. GTPs must be able to efficiently perform, validate and innovate the processes. GTPs are willing to share their expertise with other platforms.

consultation with the responsible researcher and the Animal Welfare Body (IvD). The purpose of this is to guarantee the genetic integrity, quality and continuity of the GM line and to tailor the breeding population size to the number of animals required for experimental purposes.

Appendix 2 describes the considerations that should play a role in the choice of stages for creating and breeding a GM line and the aspects and quality inspections that play a role in both cryopreservation and rederivation.[6]

The strategy described for cryopreservation (and rederviation) should form an integral part of breeding programme coordination.

4.5 Initiate a social dialogue on the application possibilities of "gene editing".

Also in light of the societal sensitivity of these issues, the NCad recommends initiating a social dialogue on this technological development similar to the "Nanopodium".[7]

In addition, when defining the target situations as described in the NCad advisory report "Transition to non-animal research methods" of 15 December 2016, the researchers concerned should consider the application of this technology, particularly for "higher" animal species, from the perspective of their moral responsibility as well as from a scientific perspective.

[6] Cryopreservation is the storage of biological material (for example embryos and sperm) at a very low temperature (−196oC).
Rederivation is the process used to obtain animals with the desired microbiological status for further breeding and use.

[7] Final report of the Committee on the Social Dialogue on Nanotechnology, *"Verantwoord verder met nanotechnologie"* [Responsibly advancing nanotechnology], findings of March 2009–January 2011; **https://zoek.officielebekendmakingen.nl/blg-98968**

5. Substantiation of the advisory report

A number of the recommendations contained in the first advisory report, "Genetically modified animals killed in stock", have been implemented in practice since this report was published by the NCad.

The method used to report animal procedures and laboratory animals in *Zo Doende*, the NVWA's annual review of animal procedures and laboratory animals, was adjusted with effect from 2014. In line with the NCad's recommendation, the registration categories for animals killed before use in an animal procedure are now more clearly differentiated:

- Disease or other health-related problems
- Ex-breeding animals, unsuitable for scientific purposes
- Unsuitable for the experiment due to age
- The "wrong" gender for the experiment
- Unsuitable genetic makeup for the experiment

This differentiation in categories had not been made, or at least not to a sufficient extent, in previous registration years. The NVWA reported the animals that had been bred but had not been used in animal procedures under the heading "died or killed in stock". This was a catch-all label for animals that had not been used in an animal procedure for various reasons.

Since various categories are now being registered, it is possible to provide greater insight into the effects of quality criteria across the entire chain for the generation of GM animals up to the breeding of suitable experimental animals.

The underlying reason for killing an animal has become clear due to the differentiation in categories.

5.1 Advise the Central Authority for Scientific Procedures on Animals (CCD) to follow the Codes of Practice (CoP) as included in Appendices 1 and 2 as the basic principles for granting project licences.

The CoPs included in the appendices were drawn up by two working groups in accordance with the latest insights.

The NCad believes that in cases involving genetic manipulation, the process of introducing the modification and the breeding of the animals concerned should be carried out with the greatest possible care, and the breeding population size should be tailored to the number of animals actually required for an experiment. This recommendation therefore aims to contribute to reducing and refining the generation and breeding of GM animals.

The NCad considers it essential that institutions should either possess or have access to the knowledge and resources required to make full use of this CoP.

5.2 Involve "centres of excellence for genetic engineering" in both planning and generating of GM lines.

A researcher or institute should possess or have access to specialist knowledge of every stage in the process of generating an GM animal model. Specialist knowledge combined in a formal or loose organisational structure could be called a Gene Technology Platform (GTP). This is a constantly evolving specialist team that performs research, creates innovations, exchanges knowledge and has the infrastructure designed for the application of the techniques at its disposal.
A GTP may be centralised in a single facility, in which all procedures are performed by the same team, or have a decentralised organisation, in which molecular biological, embryological and animal experimentation expert teams jointly constitute the platform.

GTPs should have staff with sound and detailed knowledge and experience to be able to advise researchers about the most suitable method for generating the desired GM strain and how the GM strain should be bred once it has been created. GTPs will need to have the knowledge, expertise and infrastructure or resources available to create and characterise GM animals according to the latest insights. GTPsmust be willing to share their expertise with other platforms and with any new initiatives that will be launched.

The researchers determine which GM animal model they require for their research. If it has been established that the model required is unavailable, it can be created by a GTP on the basis of a licence granted by the Central Authority for Scientific Procedures (CCD). The researchers and the GTP determine which genetic modification(s) should be introduced and the most suitable technique(s) for doing so. Scientific research institutions already have transgenic facilities that can be labelled as a GTP. In addition, various commercial businesses generate tailor-made GM animal models to order.

5.3 Let the Gene Technology Platforms (GTPs) provide an overview of the available GM lines.

A decision to create a GM animal model should only be made if it has been ruled out that the model has not already been generated elsewhere and is available. There are various international databases in which the GM lines of mice and rats are recorded, such as the International Mouse Strain Resource (IMSR; **http://www.findmice.org/**), the International Mouse Phenotyping Consortium (IMPC; **http://www.mousephenotype.org/**) and Rat Resource & Research Center (**http://www.rrrc.us/**), in addition to databases for mouse genes – Mouse Genome Informatics (MGI; **http://www.informatics.jax.org/**) – and rat genes – the Rat Genome Database (**http://rgd.mcw.edu/**).

The research community should undertake efforts to report the genetic modifications created to MGI and the GM lines to IMSR. Furthermore, it is important to be able to consult at least the GM animal models available in the Netherlands, for instance, using a system similar to Mouse locator-UK.[8] The GTPs and animal testing

[8] Bugeon L, Rosewell I. 'Mouse locator-UK': a networking tool for academic transgenic research in the UK. *Transgenic Res* 2003;12(5):637.

facilities are well-positioned to set up such a system and put it into operation.

5.4 Assign the task of coordinating the breeding programme to the animal testing facility and ensure that the cryopreservation and rederivation strategy form an integral part of this task.

Many institutes have implemented the NCad's recommendation from its first report to appoint or assign one or more breeding coordinators. However, further attention should be paid to the actual content of the task of "breeding programme coordination".

Depending on the organisation within the institute, this task can be implemented in various ways depending on the steps in the chain performed within the institute for the generation and breeding of GM animals. The generation, acquisition and/or breeding of GM animals will determine which specific knowledge and expertise should be available at the animal testing facility in order to advise and support researchers and the Animal Welfare Body (IvD) according to the latest insights.

5.5 Initiate a social dialogue on the application possibilities of "gene editing".

Gene-editing techniques also enable genetic modifications to be introduced into animal species other than mice. **See also the dilemma described in Section 3.**

Appendix 1: *Code of practice for the generation of genetically modified animals*

1. Introduction

The number of GM animal models is rapidly increasing across the globe. In general, these animals are available to researchers who wish to use them. This means that there is no need to repeatedly develop, create and characterise known GM animal models anew and connection with scientific literature is ensured. In the event a required GM animal model does not exist, is unavailable and cannot be effectively developed by cross-breeding with existing models either, it may be considered to generate the model. The justification for doing so should be clearly substantiated. The process steps set out below aim to offer guidance on providing the required substantiation.

The generation of a GM animal model takes place in a number of stages:
The scientific researcher who needs a new GM animal model for a particular research question is the decision maker in terms of DNA sequences and types of modifications that should be introduced for the purpose of addressing that question. The researcher is required to substantiate the need to generate a new GM animal model based on a Synthesis of Evidence[9] in connection with an application for a project licence or a research plan under an existing project licence for the generation of GM animals. The application for generation of the GM animal model should subsequently be submitted to a Genome Technology Platform (GTP).[10] In consultation with the GTP, the most efficient modification technique/method is selected that is likely to have the greatest chance of successfully introducing the modifications into the genetic background required for the research project.

[9] Synthesis of Evidence in laboratory animal research, NCad Opinion, March 2016

[10] A Genome Technology Platform (GTP) is a core facility, either public or private, which possesses the knowledge and expertise to supervise and perform the entire process from design to the creation of a GM animal model. GTPs must be able to efficiently perform, validate and innovate the processes and to share their expertise with other platforms.

2. Preparation/Intake

2.1 Is a suitable GM animal model already available?

Check whether the line with the desired genetic modification on the correct genetic background exists somewhere in the world and can be supplied. This not only has the advantage that the line will not need to be regenerated but that the genotype, phenotype and applications in research will also be available.

Approach:

- Consult databases such as www.infrafrontier.eu, **www.findmice.org**, **www.jax.org**, **www.mousephenotype.org**, **www.komp.org**, **www.rrrc.us** and **www.rgd.mcw.edu** as well as peer institutes and scientific literature. It would be advisable to involve a GTP in searching the databases and consulting expert networks.
- If the GM line is available, import it in the form of breeding animals, sperm or embryos. Depending on the import policy of the recipient animal testing facility, the animals or material may have to be received and kept in quarantine, and a rederivation procedure may have to be completed to avoid the introduction of undesirable micro-organisms.
- If a GM is available with the desired genetic modification but cannot be supplied on the desired strain background, consider whether it would be preferable to regenerate the model or to backcross the existing GM animal model on the desired background. The chances of success, the time investment and the number of laboratory animals to be used should all be considered in this context.

2.2 Has the Central Authority for Scientific Procedures (CCD) issued a project licence for generating the GM animal model?

Under the Dutch Experiments on Animals Act (*Wet op de dierproeven*), GM animals may only be generated if the competent authority has issued a project licence. Since the generation of a GM animal model is not an objective in itself, the application for a licence to generate GM animals should always be within the context of the research objective.

Approach:

- Consult the CCD website for current project licence application forms and the accompanying instructions.
- GTPs may also apply for a broad project licence for the generation of new models. Each model that is to be generated should be part of the research project of the end user who applies for the project licence. The advantage is that the generation methods described fall under the responsibility of the GTP licence holder and are described in a uniform manner. The Animal Welfare Body (IvD) of the institution where the GTP is located verifies the specific approach for each application.

3. Identify the characteristics of the new GM animal model

3.1 Based on the SoE referred to earlier, determine the type of genetic modification that should be introduced.

This is determined by the research question. The options range from over-expression to partial, time-restricted or complete inactivation ("transgenes", "knockdown" and "knockout") of one or more genes and relate to the choice for the most suitable location(s) in the genome for introducing the desired genetic modification(s) ("transgenes" and "knockin").

3.2 Determine how the genetic modification should be expressed. The following choices should be made:

whether the genetic modification should always be expressed or only from a given moment (constitutive versus conditional);

- whether the genetic modification should or may be expressed in all cells of the body or only in certain cell types or organs (constitutive versus conditional);
- whether expression should take place under certain conditions and whether the expression of the genetic modification should be able to be activated and deactivated (conditional versus inducible).

3.3 Determine which additional characteristics should be linked to the genetic modification.

Genetic modifications do not always result in clearly visible or recognisable functional changes. In such cases it may be necessary and/or desirable to introduce "markers" together with the modification of the gene(s) of interest. The presence or absence of the "marker" protein will be indicative of the expression of the genetic modification.[11] The presence of a "marker" may also be required if there is a clear phenotype or to be able to monitor any modification of the expression of a certain gene or certain cells in the animal.

3.4 Determine the desired genotype of the GM animal model for use in the animal experiment and the required genotype of the breeding animals if a "pure" (homozygous) line cannot be bred for reasons of animal welfare or breeding productivity.

Determine whether the genetic modification(s) should be present in homozygous or heterozygous conditions and whether this is different for breeding the GM line and for the GM animals used for the animal experiment. The choice co-determines the route to be followed for introducing several modifications. If there are multiple suitable methods with the same degree of distress, the reduction of the number of animal procedures (animals in experiments) and "died or killed before being used in a breeding programme or animal procedure" should be the guiding principle.

[11] GM lines are also created only with expression markers without any functional changes.

3.5 Determine the genetic background on which the GM animal should be generated.

The genetic background on which the genetic modification is introduced co-determines the phenotype of the GM animal model. This is why the choice of genetic background is determined on scientific grounds.

If the desired genetic modification is already present in a GM strain that is available but has an unsuitable genetic background for achieving the research objective, it should be considered to backcross the genetic modification to the desired genetic background or to reintroduce the genetic modification into the desired background. In this assessment, the envisaged quality of the final result and the inevitable number of animals died or killed for use in breeding programmes or animal procedures serve as the guiding principle.

4. Design and production

When the required characteristics of the new model have been defined, the most suitable method for introducing the genetic modification can be determined and the molecular construct (vector) that is required to create the genetic modification can be designed.

This involves a number of process steps that depend on the technological state of the art – which is constantly evolving. A GTP has staff with the knowledge and expertise required to generate GM animals according to the latest insights. This is why this CoP does not include a summary of the process steps.

The correct name of each GM line generated should be determined in accordance with the international rules for nomenclature (**https://www.jax.org/jax-mice-and-services/customer-support/technical-support/genetics-and-nomenclature**).

Appendix 2: *Code of Practice for breeding GM animal models*

1. Breeding programme coordination

The coordination of the breeding programme entails breeding laboratory animals with the aim of implementing and monitoring the specific breeding strategy for the strain that is to be bred for the purpose of producing animals for the preservation of the strain and for use in animal experiments. Breeding programme coordination ensures that the correct nomenclature and names are used for the breeding lines in accordance with the international rules for nomenclature (**https://www.jax.org/jax-mice-and-services/customer-support/technical-support/genetics-and-nomenclature**). The breeding strategy focuses on monitoring the genetic integrity of the strain, the welfare of the animals and the prevention of avoidable surplus breeding. Cryopreservation and rederivation form an integral part of the breeding strategy. Breeding programme coordination monitors the data on breeding within the institute and the use of the animals produced for experiments, and analyses and assesses these data according to the registration of laboratory animals bred. Breeding programme coordination formulates the breeding strategy based on this information, or adjusts an already implemented breeding strategy, where necessary. Depending on the type of institute and its remit, breeding programme coordination should be assigned to one or more people. Breeding programme coordination advises researchers and the Animal Welfare Body (IvD) on the breeding programme, and assists them in managing it and carrying it out.

2. The acquisition of GM animals

A project licence must be available if a GM line has a harmful phenotype. A line with a harmful phenotype is a line in which the welfare of all or certain animals has been compromised due to their genetic makeup, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

Breeding the GM line may form part of a project licence with a specific research objective. It is also possible to use a broad project licence for the purpose of breeding GM lines with a harmful phenotype.

2.1 Self-generated GM animals

As soon as a new GM line has been generated, it should be determined whether the GM line has a harmful phenotype (within the meaning of welfare impairment). To that end, the welfare of the animals should be monitored during the first two generations of the new GM line. Although a licence is not required for monitoring purposes, the guiding principle is that no activities exceeding the distress threshold should be performed in the monitoring process.

Both the European Commission and the CCD have set out directives and guidelines for monitoring the welfare of a new GM line.[12]

2.2 Imports

GM animals may be introduced into the research facility by importing:
- live breeding animals;
- embryos/sperm/ovaries/egg cells, cryopreserved or otherwise.

Preparations
- Document the transportation logistics as well as the specific paperwork required. In the case of live animals this should be carried out in collaboration with a recognised company specialised in the logistics of laboratory animal transportation.
- Ensure clear lines of communication between designated representatives of the parties involved in the shipment, transportation and receipt of animals.
- Ensure that the shipper shares the available information on the animals or embryos/sperm/ovaries/egg cells with the recipient or the recipient animal testing facility.
- Provide proof that the destination of the animals or embryos, sperm/ovaries/egg cells is known within the recipient institute or the animal testing facility.
- Document that appropriate measures will be taken to protect the integrity of the shipment and the environment upon receipt (e.g. regarding microbiological quality).

Receipt

Quarantine
- Take receipt of live animals in a quarantine environment. "New" breeding animals are only permitted to enter the breeding programme after documented verification of the desired health status. This is determined on the basis of the health status of the origin and destination colonies, and is safeguarded by microbiological screening and/or rederivation.
- Check and report the integrity of the shipment upon arrival (labels, documents, packaging).
- Check and document the animals with respect to condition, number, gender distribution, etc. and confirm that this is in accordance with the description of the shipment.
- Test the genotype of the animals before additional activities taken place.
- Ensure proper and full, centralised registration.

Cryogenic storage
- Check and document upon receipt whether the material is still in the proper physical condition (e.g. whether it is still deep-frozen).
- Upon receipt, ensure that the frozen material is and remains at the right temperature in the designated storage unit: monitor the desired temperature.
- Ensure proper and full, centralised registration.

[12] http://ec.europa.eu/environment/chemicals/lab_animals/pdf/corrigendum.pdf https://www.centralecommissiedierproeven.nl/documenten/formulieren/16/10/13/handreiking-kaders-genetisch-gewijzigde-dieren

2.3 Purchase

Establish and document the policy for dealing with animals from legally recognised suppliers. A distinction can be made between animals directly intended for use in experiments and animals intended for breeding.

3. Breeding GM animals

The breeding strategy for each GM line should be tailored to the use of the GM line in the research project, the complexity of the desired genotype (including the genetic background) and whether or not the expression of a phenotype affects reproductive biology.

3.1 Breeding strategy

- Make a distinction between breeding for maintenance, expansion and production purposes.
- Make a distinction between breeding GM lines with distress levels exceeding the threshold and breeding GM lines without distress levels exceeding the threshold, and document the information. A project licence from the competent authority is required for an animal procedure that involves breeding a GM line with distress levels exceeding the threshold.
- Avoid maintaining a GM line in a breeding programme if there are no prospects for its use in an experiment. If the line remains available elsewhere, the breeding programme should be discontinued. If the particular line is unique or a cross-breed and the research results have been published, the line should remain available, also for third parties. Cryopreservation is the appropriate solution if the line would otherwise be maintained in the breeding programme for a prolonged period without using the animals bred for experiments. The cryopreservation of embryos can be performed most efficiently when the line is still being produced in the breeding programme.

- Avoid the unnecessary breeding of generally, commercially available GM strains. There may be legitimate reasons however for setting up your own breeding programme for such a strain, such as animal welfare and the special characteristics of the animals used.
- Terminate the breeding programme of a GM line with which scientific results have been achieved and published once it is clear that sufficient representative genetic material in the form of embryos and/or sperm and/or ovaries and/or ES cells has been stored in an accessible cryo bank.
- Coordinate breeding for expansion and production purposes with the planned take-up of animals (according to genotype, gender and age) for use in experiments. Use calculation models to estimate the minimum size of the breeding population for expansion or production purposes.[13]
- Consult information sources, such as:
  - own data on the number of animals produced in breeding programmes;
  - the general parameters for the reproduction of the background line;
  - information on any deviations from a Mendelian distribution of genotypes in the offspring.

[13] Breeding strategies for maintaining colonies of laboratory mice: a Jackson Laboratory Resource Manual (**www.jax.org**).
Efficient breeding of genetically altered animals: assessment framework (**http://org.uib.no/dyreavd/Documents/GAA%20tool.pdf**)
The Jackson Laboratory Handbook on Genetically Standardized Mice. Bar Harbor, Maine, USA: The Jackson Laboratory, 2009.

3.2 Cross-breeding and backcrossing

Cross-breeding and backcrossing contribute to the number of animals sacrificed before use in breeding programmes or animal procedures.

- It may be more efficient to generate the desired animal model in another manner; consult a GTP for this purpose. Breeding programme coordination ultimately determines whether cross-breeding or backcrossing can be avoided.
- If, in order to backcross a WT strain, a crossing partner is required which is generally commercially available, animals may be purchased specifically for that purpose. If, however, there are good reasons for setting up your own breeding programme for such a strain, avoid the formation of substrains by replacing the animals with newly acquired animals from the same source or supplier within a maximum of 10 generations.

3.3 Breeding programme records:

The results of breeding a GM line can only be optimised if breeding data are continuously collected, documented and analysed in a systematic manner at a centrally accessible location.

- The following data should at least be recorded for this purpose:
  - the origin of the line;
  - the genotype and the genetic background of the strain;
  - the phenotype of the strain (characteristics and deviations observed);
  - the family tree;

- the breeding programme yield (period between litters; number of litters per female, number of pups weaned per litter; gender ratio per litter; genotype distribution).

3.4 Rederivation

Rederivation is the process that is used to obtain animals with the desired microbiological status for further breeding and use. Rederivation can be performed by means of peripartum hysterectomy and subsequent transfer of the pups to a "clean" foster mother who has just given birth, or by means of embryo transplantation. In embryo transplantation the embryos of donor animals are introduced, often after superovulation, into the recipient animals who first undergo a pseudo-pregnancy by pairing with a sterile male. Rederivation is used if imported animals do not have the required microbiological status for the procedures and/or the animal facility. Rederivation should preferably be performed after ascertaining that the imported animals have the desired genotype and that the GM line will be used for a prolonged period. The recipients of the transplanted embryos should at least have the same health status as that required for the procedures and the animal facility and should be suitable for microbiological screening.

- Determine the required microbiological status. Consider factors such as international recommendations, the requirements imposed by the research programme and the facility's infrastructure. Every facility should have a health monitoring programme in place for the animal populations present (*Annex III Directive 2010/63/EU*).
- Assess the report on the microbiological status of the animals that are to be imported or the shipment in the context of the health monitoring programme for the colony from which the imported animals originate.
- For single homozygous GM lines, it would be preferable to import males of the GM line. For multiple GM lines, it would be advisable to import both males and females, to cross them and to collect the embryos for transplantation.
- Use females with the desired genetic background as embryo donors.
- Verify the microbiological status of the rederived animals before including them in the colony. This can be done by microbiologically screening the foster mother. Pending the results, the animals should be housed separately from the destination colony.

3.5 Cryogenic storage

- Determine whether a GM line should be stored. Answer the following question for this purpose: Is the (modification in a particular genetic background) line unique, has it already been stored elsewhere, is it still of scientific interest or can the line be "easily" recreated?
- Determine the material that is stored from a GM line based on the number of genetic modifications and the status of those modifications to the GM line and breeding efficiency.
- Ensure complete documentation and registration of the frozen material, the freezing method and the recommended thawing method.
- Incorporate a number of temperature checks (i.e. is material still frozen?).

- Check the vitality of each frozen GM line.
- Ensure that the stored material of a GM line is at least equally distributed across two locations physically separated from each other so that, in the event of an emergency, half of the material is still available at one location at least.

Embryos

As an embryo contains the double set of chromosomes (diploid genome) or the complete genome of the GM line, this approach is preferred for the cryogenic storage of GM lines with multiple genetic modifications. A minimum of a few hundred to as many as 500 embryos will usually be frozen per line. This requires a considerable number of donor mice. To obtain many embryos from a donor, superovulation can be applied by treating (prepubertal) females with hormones.

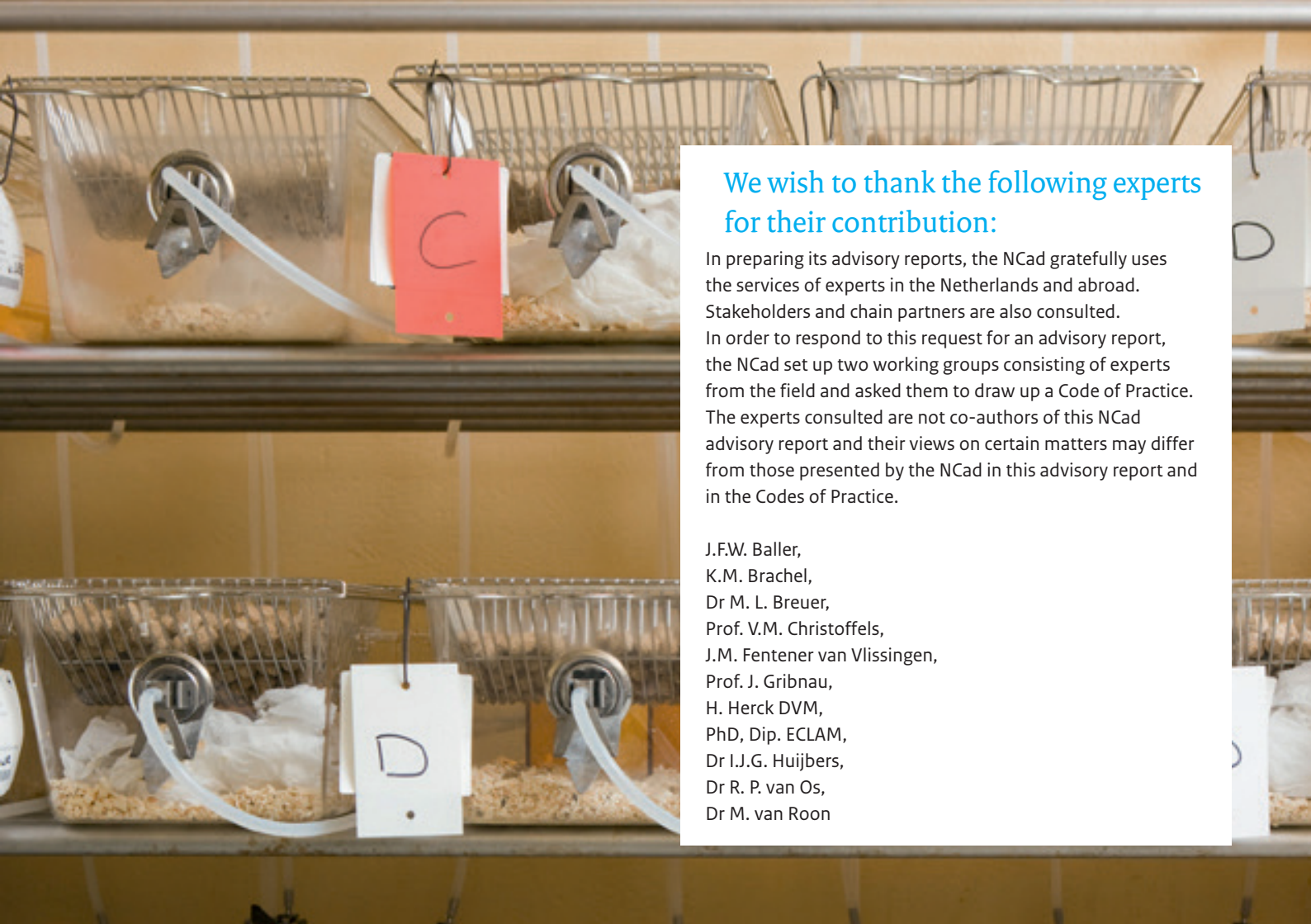
Cryopreservation of gametes

Male (sperm) or female gametes (eggs and ovarian tissue) only contain a single set of chromosomes (haploid genome). After thawing, sperm is combined with fresh or thawed egg cells (usually from the wild-type background line) and thawed egg cells with fresh or thawed sperm (usually from the wild-type background line). The resulting embryos are implanted into pseudo-pregnant recipient animals. Thawed ovarian tissue is transplanted into a recipient that is subsequently covered by a male of the desired background line. The cryopreservation of gametes is particularly suitable for the cryogenic storage of GM lines with a single genetic modification. In the case of a combination of modifications, following the revival of the line the breeding programme should focus first and foremost on obtaining the desired breeding animals, preferably homozygotes but, if necessary, heterozygotes.

Embryonic stem cells with the desired modification

This approach can only be used as a viable alternative for the refrigeration of embryos and gametes if the genetic background of the stem cells is the same as the desired background of the GM animal model. If this is not the case, backcrossing will be inevitable (**see 3.2**). This requires a lot of time and many extra animals. In such cases, embryos, sperm or ovarian tissue should be frozen.

Notes



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In order to respond to this request for an advisory report, the NCad set up two working groups consisting of experts from the field and asked them to draw up a Code of Practice. The experts consulted are not co-authors of this NCad advisory report and their views on certain matters may differ from those presented by the NCad in this advisory report and in the Codes of Practice.

J.F.W. Baller,
K.M. Brachel,
Dr M. L. Breuer,
Prof. V.M. Christoffels,
J.M. Fentener van Vlissingen,
Prof. J. Gribnau,
H. Herck DVM,
PhD, Dip. ECLAM,
Dr I.J.G. Huijbers,
Dr R. P. van Os,
Dr M. van Roon

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PO Box 93118
2509 AC Den Haag
0900 2800028
NCad@minlnv.nl
www.NCadierproevenbeleid.nl

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The NCad was founded to protect animals used for scientific and educational purposes. The NCad achieves visible improvements in the Replacement, Reduction and Refinement (3Rs) of animal procedures and the ethical review thereof in order to minimise the use of laboratory animals, both nationally and internationally. The ethical review of animal procedures and the 3Rs are central to this aim.