



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

Genetically modified animals killed in stock

Advisory report, prepared by members of the
Netherlands National Committee for the protection of
animals used for scientific purposes (NCad) at the request
of the Minister for Agriculture



The NCad and its methods

The Netherlands National Committee for the protection of animals used for scientific purposes (NCad) was appointed for the protection of animals used for scientific and educational purposes. NCad aims to make a significant contribution to minimising laboratory animal use, both at national and international level. This will involve giving advice, exchanging knowledge, and developing both national and international networks. The ethical review of animal procedures is of pivotal importance in this regard, as are the 3Rs (Replacement, Reduction and Refinement).

Members of the NCad

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Summary

Animals killed in stock does not only imply surplus animals from genetic modification projects: approximately 25% of the animals killed before the procedure are non-genetically modified animals. The substantial increase of animals killed in stock is primarily due to experiments with zebrafish and mice.

The main reason for killing these animals is that they have an unsuitable genetic composition. Other reasons include age, weight, or incorrect gender for the research purposes.

The present advisory report focuses primarily on reducing the number of genetically modified (GM) animals that “died or killed in stock”, especially fish and mice. However, the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) also draws attention to other aspects that it considers relevant to the dynamics of the numbers and species of GM animals used. It also places the issue of the number of animals killed in stock in a broader perspective. Recent years have seen the development of “genome editing”, a new and innovative technology that can be used to create genetically modified animals. Genome editing is increasingly replacing traditional transgenic technology. This new technology is expected to make it possible to create a genetically altered animal that is tailor-made for a given experiment while using fewer animals in the process than has hitherto been the case. The advisory report builds on previous initiatives by the Ministry of Economic Affairs. Given the inexorable march of scientific and technological progress, it is advisable to have this advisory

report periodically evaluated by the NCad and, if necessary, modified. Specifically, the NCad recommends the Minister of Agriculture

Quality criteria

- to provide a further boost to the virtual centralisation between licenced establishments that is now under way, and to promote such cooperation through the research agenda. The NCad concludes that concentrating the generation of GM animals in so-called “centres of excellence” (in full compliance with this advisory report) will have no added value;
- to oblige every licenced establishment where GM animals are bred to appoint one or more suitably qualified breeding coordinators. To this end, a compulsory training module should be formulated that is tailored to the post in question;
- to instruct the Netherlands Food and Consumer Product Safety Authority (NVWA) to clarify the shifts in laboratory animal numbers and animal procedures from one registration year to another, as a result of the changed registration system. The NVWA should also be instructed to replace the category of “died or killed in stock” with the official name of the registration categories, in “Zo doende” (the NVWA’s annual review of animal procedures and laboratory Animals

Efficiency criteria

- to instruct the research groups or facilities to submit, no later than the end of 2016, a report in which the expected reduction of laboratory animals is substantiated on the basis of national and international empirical evidence. This report should also indicate the maximum number of laboratory animals that may be used for a

single genome-editing experiment, to generate a required animal model;

- to only permit the generation and breeding of GM lines if the licenced establishment has access to a state-of-the-art programme for the cryogenic storage of embryos and/or sperm, and for the revitalisation of frozen material;
- during the assessment and ethical review of project licences by the Central Authority for Scientific Procedures on Animals (CCD), instruct the CCD to routinely take into account whether, in the project application, the researcher in question has clearly shown that he/she has fully explored the aspect of animals that “died or were killed before being used in breeding programmes or animal procedures” and has demonstrated that he/she has kept the number of animals used to a minimum. The guiding principle must be that, with regard to the gender principle, both genders should be included in the procedure’s design. In addition, the selected age limits or weight limits should not be unnecessarily restrictive;
- to ask the research groups or facilities that are pioneering the development and/or implementation of new genetic modification technologies to introduce additional criteria and quality standards (under the direction and control of the NCad), including a significant reduction in the number of animals required to generate GM animals.

The NCad points out that genome-editing technology also makes it possible to generate GM animals from other, “higher” species than the mouse and the zebrafish. As a result, laboratory animal use could increase. That would be at odds with the social desire to further reduce laboratory animal use.

The NCad, therefore, believes that if the problems associated with the breeding of genetically modified animals are to be solved, then a comprehensive approach aimed at a net reduction in laboratory animals will be required.

Thus, in conclusion, the NCad’s final recommendations are as follows:

- With regard to the moral responsibility of those involved, to initiate a timely, open debate on the implementation of “transgenic technology” in higher animal species, based on specific cases. The ultimate aim here is to establish policy frameworks, possibly in a European context

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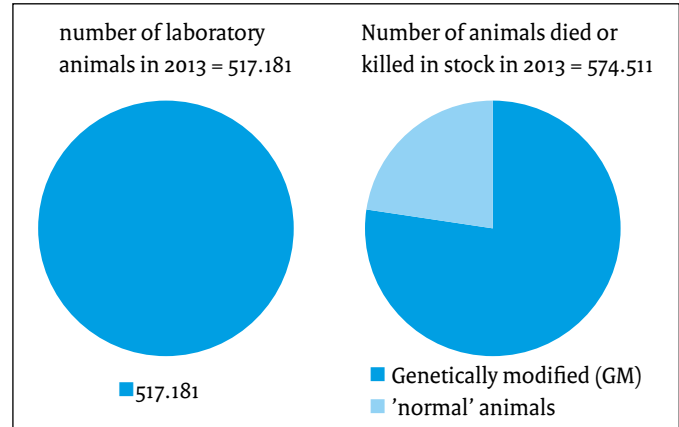
1. Introduction

Animals used in animal experiments are often bred for that specific purpose. To date, more animals have been bred than are used in animal experiments. Any unused animals are killed. Over the past ten years, there has been a sharp rise in the number of animals that are killed before the procedure.³ Indeed, in 2013, this number exceeded the number of animals that were actually used in experiments. This situation raises moral questions.⁴

Both GM and non-GM animals are killed in stock. Less than 25% of the animals killed before the procedure are non-GM animals.

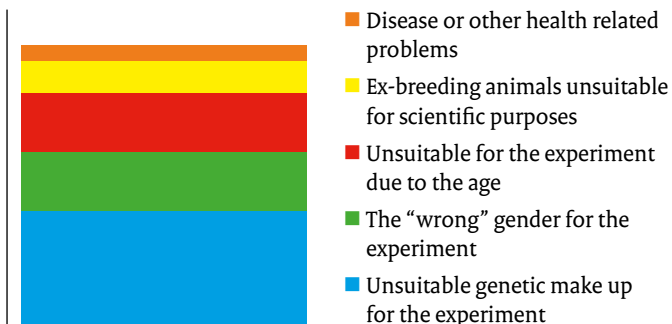
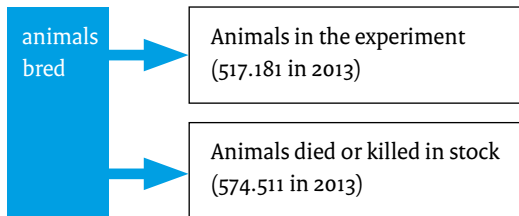
Thus, the majority (more than 75%) are GM animals. The sharp rise in the number of animals reported by the Netherlands Food and Consumer Product Safety Authority (NVWA) as “died or killed in stock” over the past ten years is restricted entirely to GM mice and zebrafish. The factors that render GM animals unsuitable for experiments (i.e. to be reported as “died or killed in stock”) include a genetic composition that is unsuitable for the experiment in question, age (e.g. “too old”), weight (e.g. “too heavy”) or they may be of the wrong gender (see also the section on the NVWA’s annual registration and annual report).

GM technology, together with knowledge of the human and mouse genomes, have made it possible to bring about targeted changes in the mouse genome to create representative models for human diseases. In accordance with the laws of genetics, breeding GM animals that have one or more modified genes will produce some progeny that do not have the desired genetic composition. Such animals are, therefore, killed before the animal procedure. Increases in the use of GM animals



(particularly in biomedical research) and in the number and range of complex hybrids, plus advances in the technology for creating GM animals, account for the increase in the number of animals categorised as “died or killed in stock”. The extent to which this increase is justified by scientific output and relevance to human and animal health is the subject of debate.

Genome editing, a new and innovative technology that can be used to create GM animals, is increasingly replacing traditional transgenic technology. It also makes it possible to produce, by relatively simple means, a variety of GM lines that are potentially very useful in terms of research. Experience gained to date shows that the number of animals previously required to create a GM mouse strain can be significantly reduced by means of genome-editing technology.

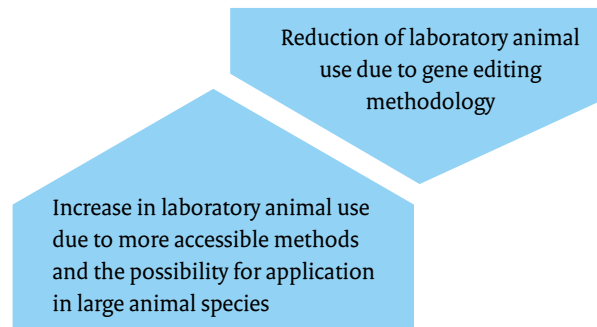


Animals died or killed in stock

The breeding programmes needed to obtain a stable line, and the associated characterisation experiments, will be registered as animal procedures. "Genome-editing" technology makes it possible to create GM mice, irrespective of their genetic background or strain. Indeed, multiple modifications can be created simultaneously. As a result, there is little or no need for many of the above-mentioned complex

hybrids. Accordingly, they will contribute little or nothing to the number of animals categorised as "died or killed in stock". Genome-editing technology also makes it possible to generate GM animals from other, "higher" species than the mouse and the zebrafish.

The situation described above provides opportunities for reducing the number of animals categorised as "died or killed in stock". It can also lead to better laboratory animal models for human diseases and for expanding scientific knowledge. A potential downside, however, is that laboratory animal use may increase, partly as a result of the application of this technology in additional species (Hendriksen & Spielmann, 2014) (Combes & Balls, 2014).



As a project licence is required for each genome-editing experiment, the Central Authority for Scientific Procedures on Animals is in an excellent position to identify trends and areas of application. In view of the research objective, this advisory report focuses solely on

reducing the number of GM animals categorised as “died or killed in stock”, especially fish and mice. The NCad will further explore other aspects that it considers relevant to the dynamics of the numbers and species of GM animals used for scientific purposes.

2. Request for advice

In her request for advice, of 31 March 2015, the Minister for Agriculture (EZ) asked the NCad to establish: “more exhaustive efficiency criteria and quality criteria for the generation of genetically modified animals in centres of excellence”. “These criteria should promote quality in the breeding of genetically modified animals in the Netherlands and make breeders aware of a possible reduction in the total number of animals used for this purpose. It is recommended that these criteria initially be used for the in-house generation of genetically modified mice and fish (using both traditional and innovative methods).”⁶

In this connection, the Minister for Agriculture has asked the NCad to take the 2014 Regular Consultation Body on Animal Procedures and Alternatives (RODA) advisory report into account, as well as the results of the international workshop “Bred but not used”, and the 2011 advisory report from the former Central Authority for Scientific Procedures on Animals (CDD) on the centralised breeding of transgenic animals.^{7, 8, 9}

3. Advisory report

The request for advice cannot be dissociated from the broader issues identified in the introduction. The advisory report also makes some recommendations in this regard.

Accordingly, the NCad advises the Minister for Agriculture and all parties in the laboratory animal chain to make every effort to bring about the careful use and further development of this technology within the frameworks indicated below.

In addition to the possible effects of the rapid technological developments in question, this advisory report explores the possibility of a better match between the supply of, and demand for, animals. This advisory report makes a number of recommendations on how additional quality assurance can be achieved, and about efficiency criteria to reduce the number of animals killed in stock.

The efficiency criteria also provide guidelines for the assessment of project applications by the Central Authority for Scientific Procedures on Animals (CCD). These guidelines take the animal’s entire life into account, whether or not it has been genetically modified.

The advisory report builds on previous initiatives by the Ministry of Economic Affairs. Given the inexorable march of scientific and technological progress, it is advisable to have this advisory report periodically evaluated by the NCad and, if necessary, modified. An initial evaluation could coincide with the publication of the NVWA’s 2016 annual report (in mid 2017).

3.1. Quality criteria

3.1.1 Virtual Centralisation

The NCad recommends that a further boost be given to the virtual centralisation now under way (in which licenced establishments exchange data on available GM lines in breeding programmes, as frozen embryos and/or semen).⁴⁰ It recommends that such cooperation be promoted via the research agenda. The NCad concludes that concentrating the generation of GM animals in so-called “centres of excellence” (in full compliance with this advisory report) will have no added value. Accordingly, it adopts the conclusion of the former CCD’s 2011 advisory report.

3.1.2. Monitoring of technological developments

Genome-editing technology is increasingly being used to generate GM animals. This technology is expected, eventually, to replace traditional transgenic technology (Skarnes, 2015). The NCad recommends that the implementation and further development of this technology should be subject to continuous monitoring, involving regular progress reports. This will make it possible to explore its impact on the generation and breeding of GM animals, including the number of animals that are killed before the procedure. To this end, an assignment can be awarded to a research institute, working in close cooperation with the NVWA (as a data collector).

Genome-editing technology also makes it possible to generate GM animals more quickly and easily from species other than the mouse

and the zebrafish. The generation of GM animals requires a project licence. The CCD will only issue such a licence after assessing a detailed ethical review prepared by an accredited Animal Ethics Committee (DEC).

In addition, the NCad points out the moral responsibility of those involved, and advises the Minister for Agriculture to initiate a timely, open debate on the implementation of transgenic technology in higher species, as soon as specific, relevant cases become available. The ultimate aim here is to establish policy frameworks, possibly in a European context.

3.1.3. Augmenting breeding management – breeding coordinator

The breeding of GM animals is the joint responsibility of the manager of the facility in which the breeding programmes take place and the researcher or owner of the GM lines in question. To safeguard quality and efficiency, the NCad advises the Minister for Agriculture to oblige every licenced establishment where GM animals are bred to appoint one or more suitably qualified breeding coordinators. To this end, a compulsory training module should be formulated that is tailored to the post in question. Breeding coordinators should have sufficient means at their disposal to manage breeding programmes as effectively as possible. Breeding coordinators must adequately inform the Animal Welfare Body (IvD) regarding the status of breeding programme management in each of the lines (including GM lines) within the licenced establishment in question.

The NCad advises the Minister of Agriculture to oblige every licensed establishment where GM animals are bred to appoint one or more suitably qualified breeding coordinators

3.1.4. NVWA - annual registration and annual report

The NCad advises the Minister for Agriculture to instruct the NVWA to clarify the shifts in laboratory animal numbers and animal procedures, per registration category, from one registration year to another, as a result of the changed registration system. It is of great importance to be able to determine whether or not there is a genuine reduction in the number of animals killed before the procedure, or whether at least some of these animals have simply been shifted to other registration categories. Data from the 2016 reporting year is expected to be sufficiently stable for the purposes of an initial evaluation of this kind.

In addition, the innovations in – and the extremely rapid development of – the technology for generating GM animals may impact the dynamics of both the generation and breeding of such animals.

To clarify matters, the NCad advises the Minister for Agriculture to instruct the NVWA to include the grounds of “died or killed in stock” (for reasons such as unsuitable genetic makeup or age/weight/gender) in its annual registration. In addition, it should analyse the quantifiable differences in numbers between the new method and the traditional method, in terms of numbers of animals killed in stock.

The NCad also recommends that, in “Zo doende” (the NVWA’s annual review of animal procedures and laboratory animals), the category name “died or killed in stock” should no longer be used. Instead, the names used should correspond to those of the

categories under which the NVWA requires establishment licensees to register such animals. The NCad recommends that the annual report on animal procedures and laboratory animals should be supplemented with guidance notes, explanatory notes, and an interpretation that are intelligible to the lay reader.

3.2. Efficiency criteria

3.2.1. Generating GM lines

Based on empirical evidence, the Committee on Animal Biotechnology (CAB) has determined that a specialised transgenic laboratory would require up to 150 laboratory animals to generate a single GM line using traditional transgenic methods. The NCad expects that, with the introduction of genome-editing technology, it will be possible to reduce this number substantially in the near future. The NCad advises the Minister for Agriculture to instruct the research groups or facilities to submit, no later than the end of 2016, a report in which the expected reduction of laboratory animals is substantiated on the basis of national and international empirical evidence. This report should also indicate the maximum number of laboratory animals that may be used for a single genome-editing experiment, to generate a required animal model (see 3.3 criteria).

3.2.2. Cryogenic storage

The NCad advises the Minister for Agriculture and the CCD to only permit the generation and breeding of GM lines if the licenced establishment has access to a state-of-the-art programme for the

cryogenic storage of embryos and/or semen, and for the revitalisation of frozen material. This means that breeding programmes for GM lines that do not need to be instantly available to resolve current scientific questions are rendered superfluous. To some extent, GM lines can be exchanged nationally and internationally in the form of frozen embryos or semen, rather than live animals. This judgement will have to be made on a project by project basis. Aside from the prevention of transport stress, factors such as the laboratory animal science and scientific impacts of the need to breed more laboratory animals will need to be taken into account. Breeding coordinators have a decisive part to play in such considerations. The NCad advises the Minister for Agriculture to launch initiatives aimed at establishing agreements on this topic, at both national and international level.

3.2.3. CCD project licences: gender and age

The NCad advises the Minister for Agriculture to instruct the CCD, during the assessment and ethical review of project licences by the Animal Ethics Committee (DEC), to routinely take into account whether, in the project application, the researcher in question has clearly shown that he/she has fully explored the aspect of animals that “died or were killed before being used in breeding programmes or animal procedures” and has demonstrated that he/she has kept the number of animals used to a minimum. This advice applies to GM animals and non-GM animals alike, regardless of their origin.

The NCad feels that applicants should provide comprehensive scientific justifications for their choice of inclusion criteria for

animals for a given experiment, at least with regard to gender and to the maximum permissible age distribution. The guiding principle must be that, with regard to gender, both genders should theoretically be included in the procedure's design. This is because opting to use animals of just one gender inevitably leads to a breeding surplus of the other gender, which must, therefore, be justified.

In addition, the age limits or weight limits should be as broad as possible. When defining them, however, it should be borne in mind that the gender distribution and age distribution can lead to a greater variance in the research outcomes. Selecting unnecessarily tight limits for age or weight leads directly to an increase in the number of animals killed in stock (see the NWA's Annual Registration). The IvD and the breeding coordinator should certainly be involved in these considerations.

3.3. Criteria and quality standards - Best practices

The NCad advises the Minister for Agriculture to ask the research groups or facilities that are pioneering the development and/or implementation of new genetic modification technologies to introduce additional criteria and quality standards, including a significant reduction in the number of animals involved in the generation, breeding and use of GM animals. The NCad recommends that the large users consultative body be asked to take the initiative in this regard. It should be asked to submit a report, no later than the end of 2016, in which the expected reduction in laboratory animals is substantiated on the basis of national and

international empirical evidence. This report should also indicate the maximum number of laboratory animals that may be used for a single genome-editing experiment, to generate a required animal model; on this basis, under the direction and management of the NCad, a Code of Practice should be drawn up and subsequently presented by the NCad.

Any project applications for the generation and/or breeding of GM animals will then be assessed by the CCD on the basis of this Code of Practice.

4. Substantiation of the advisory report

4.1. Quality criteria

4.1.1. *Virtual Centralisation*

In 2011, the Minister of Health, Welfare and Sport asked the former Central Authority for Scientific Procedures on Animals (CCD) to draw up an advisory report on the issue of whether centralising the breeding of GM animals is desirable and feasible, and whether that would lead to fewer animals being killed in stock.¹¹ In its advisory report, the former CCD drew a distinction between the centralisation of breeding programmes within an individual licenced establishment and the centralisation of breeding programmes between a number of licenced establishments. After consulting the research community, it found that the breeding of GM animals largely takes place in scientific research institutes and that it is centralised within those institutes. The former CCD concluded that the centralisation of GM animal breeding programmes between a number of licenced establishments, as such, would only achieve a limited reduction in the number of animals that are killed before the procedure. Indeed, almost half the number of animals that are killed before the procedure were those whose genetic composition is unsuitable for the procedure, due to the way genetic information is inherited in accordance with the laws of genetics (see Appendix 1, NVWA annual registration). In this case, it makes no difference whether the animals are bred locally or centrally. Similarly, the technological developments outlined in this advisory report also reduce the need for centralisation. The level of quality assurance

The guiding principle must be that, with regard to gender, both sexes should theoretically be included in the procedure's design

targeted by our advisory report can be more rapidly achieved by using existing specialised laboratories, rather than by creating a central facility without the support of the research community. Accordingly, in the context of this advisory report, the NCad feels that centralisation has no added value. The NCad endorses the former CCD's suggestion that consultations between licenced establishments with breeding programmes for GM animals be encouraged, with a view to virtual centralisation (in which institutions exchange data on available GM lines in breeding programmes, as frozen embryos and/or semen).¹² Current practice with fish, in the research community, can serve as an example.

4.1.2. Monitoring technological developments

4.1.2.1. The development of genome-editing technology

The technology for creating GM animals has undergone tumultuous development over the past three to four years. The technology for making genetic changes is now so refined that individual DNA building blocks can be modified in a targeted and more or less specific way. Such genome-editing was made possible by the use of certain bacterial enzyme systems, including CRISPR-associated protein 9 nuclease derived from *Streptococcus pyogenes* (CRISPR/Cas9) (Sander & Joung, 2014). The first articles on CRISPR in bacteria were published as long ago as 2002. The use of CRISPR/Cas9 for making targeted changes to parts of the genome is a more recent development, one that has widely supplanted traditional “transgenic technology”. In addition, this technology has been very rapidly refined and improved (Fu, Sander et al., 2014). In just a short period of time, it has radically changed the

entire field of genetic research. Using this technology, it is possible to simply and very rapidly alter the genome in order to study the function of specific genes and other segments of DNA (Singh et al., 2014). To this end, animal models can be created, more easily and more rapidly than ever before, to study biological processes in “healthy” and “sick” individuals.

4.1.2.2. The application of this new technology in the mouse

Using traditional “transgenic technology”, researchers wishing to make certain genetic changes in the mouse (one of the most widely used – if not the most commonly used – species in biomedical research throughout the world) were dependent on the availability of embryonic stem cells (ES cells), to which a single specific change could then be made. Despite years of study, stable ES cell lines have only been produced from a limited number of inbred mouse strains. Accordingly, it is necessary to set up extensive breeding programmes in order to study the effects of induced genetic changes on various genetic backgrounds. The same applies to studies into combinations of various genetic changes that, until recently, could only be created by crossing GM lines that each had a single genetic change. These breeding programmes have contributed to the sharp rise in the number of animals that are killed before the procedure.

Genome-editing technology, such as CRISPR/Cas9, enables any institution with a solid grounding in the application of molecular biology and with experience in assisted reproductive technology (ART) to create GM animals with multiple genetic changes, on any desired genetic background, without the limitation of ES cell availability. If this

development continues as expected, it will reduce the need for extensive breeding programmes for the inbreeding and backcrossing of GM animals. In addition, they will make up a smaller percentage of those animals that are killed before the procedure. Due to the underlying molecular processes of genome-editing and given the state of the art with regard to this technology, it is not yet possible to guarantee that an exact copy of a GM line can be created using CRISPR/Cas9, even if the same CRISPR/Cas9 complex is used again (Hsu, Lander et al., 2014). As a result, it will be necessary to breed animals for the production of embryos and/or semen for the exchange of GM lines between licenced research establishments.

Laboratories that use CRISPR/Cas9 technology report that a single genome-editing experiment can generate numerous potentially interesting mutant lines, compared to just a few using traditional transgenic methods. Following selection on the basis of biological relevance, if the remaining lines include several stable lines that are relevant to the study in question, subsequent breeding programmes for these lines can result in an increase in the number of animals that are killed before the procedure.

Another innovative technology (developed at the Netherlands Cancer Institute (NKI) for the generation of GM lines containing several modified genes) is based on the use of molecular biological techniques to create additional genetic modifications to GM ES cells isolated from GM animals (Huijbers et al., 2011). It remains to be seen whether this technology will retain its value for research, alongside genome-editing technology.

4.1.2.3. *The application of this new technology in the zebrafish*

The increase in the number of animals killed in stock in recent years can be almost entirely attributed to GM zebrafish. Prior to the introduction of genome-editing, the methods used in genetic research on zebrafish included chemical mutagenesis, in the context of a “forward genetics” approach.¹³ To that end, male zebrafish were exposed to chemical mutagens, to introduce random mutations in sperm-cell DNA. These males were then mated to numerous females, to generate as many progeny as possible. The F₁ generation includes all of the mutations that have arisen. The progeny were then individually examined for the presence of one or more abnormalities. The DNA of animals with an abnormality was then analysed to identify both the mutation (or mutations) responsible and the associated function (Knapik, 2000). Within three years, every individual in the F₁ generation had died. A new mutagenesis experiment was then initiated, and the individuals previously used for breeding were replaced. This resulted in a significant increase in the number of animals that were killed before the procedure. With the introduction of the CRISPR/Cas9 technology, a number of researchers abandoned the use of large-scale mutagenesis experiments.

In addition, the zebrafish embryo lends itself perfectly to the introduction (by means of injection) of DNA encoding a functional gene. In this way, GM animals are generated for direct use in the study, which also helps to reduce the number of animals that are killed before the procedure.

If the implementation of these techniques does indeed replace large-scale mutagenesis experiments, then the contribution made by

zebrafish to the number of animals in the category “died or killed in stock” should decline.

However, until such time as the entire genome has been mapped in terms of functionality, the “forward genetics” approach will not be completely replaced by “reverse genetics” approaches like the genome-editing methods.¹⁴

4.1.2.4. Future areas of application for CRISPR/CAS9

The speed and extent with which genome-editing technology has been embraced and applied could lead to societal disquiet, as these developments are pushing ahead apparently without adequate societal frameworks (Ledford, 2015). The introduction of CRISPR/Cas9 technology has eliminated the limitation regarding the species in which genetic changes can be made (Niu, Shen et al., 2014). As a project licence is required for each genome-editing experiment, the CCD is in an excellent position to identify trends and new areas of application, and to draw up frameworks, if necessary. The application of CRISPR/Cas9 as a therapy for the treatment of genetic disorders in humans is a development for the future (Hsu et al., 2014).

4.1.3. Augmenting breeding management – breeding coordinator

The appointment of one or more breeding coordinators is highly relevant in the context of managing the increasing number of GM lines of potentially distinct and different species than has hitherto been the case. The university centres (including university medical centres) employ officials whose duties include breeding management. During an initial evaluation (July 2015), however, it emerged that there are major differences between institutes with regard to which officer is assigned breeding management duties and

to the way in which those duties are implemented. Every licenced establishment where GM animals are bred must be required to appoint one or more suitably qualified breeding coordinators.

The breeding coordinator must have a thorough knowledge of the breeding programme required for the species in question. He or she must be aware of the various national and international databases and available collections of GM lines, as in the context of the pan-European research infrastructure for the systematic characterisation, archiving and distribution of mouse disease models INFRAFRONTIER (Infrafrontier Consortium, 2015).¹⁵ He or she should be able to communicate effectively with animal care staff and biotechnicians, as well as with research staff ranging from PhD students to the principal investigator (PI), to take responsibility for the breeding programme, which is shared with the owner investigator.

Individuals can, to some extent, acquire these skills through experience. However, the NCad states that a compulsory training module, specially tailored to this target group, should be developed to provide the requisite theoretical knowledge in structured form.

The work of the breeding coordinator (or breeding coordinators), and hence that of the unit or facility in which the breeding programme is conducted, must be supported by a system designed to register and periodically analyse data specific to the breeding programme. The same system should preferably be used to register all the information required by law, in the context of annual reporting to the government.

In this connection, the IvD's duties include advising staff who handle animals on matters relating to animal welfare, in connection with the procurement, accommodation, care and use of animals, and with the application of the 3Rs. In addition, the IvD is expected to monitor the development and results of projects, taking into account their impact on the animals used.¹⁶ In that context, the IvD operates as a partner to the breeding coordinator, in the performance of his/her duties. The IvD will ensure that best practices are used and periodically updated.

4.1.4. NVWA - annual registration and annual report

4.1.4.1. Changed registration system

Under the revised Wod, the laboratory animal registration system has changed. The new definition of an animal procedure and its impact on registration, by means of the 2012/707 Implementation Decree and the Dutch registration booklet, can result in a change to categories such as “died or killed before the procedure” versus “died or killed during the procedure” and “died or killed after the procedure”.¹⁷ For many animals, nothing will change: both the old and the new registration systems require animals that are bred or used with a certain degree of distress/discomfort to be registered as an animal procedure. A number of animals that, before the revision, were registered in the category “died or killed before the procedure”, will be registered under the new system as “died or killed during or in the context of the procedure”. Especially when crossing and creating new genetically modified lines, the second and subsequent generations will contain more animals that are registered as animal procedures under the new registration system. This relates to chemically-induced

mutants, for example, which were not previously considered to be animal procedures. Under the new system, the crossing of two stable GM lines will also be seen as a breeding programme involving distress/discomfort (and, therefore, as an animal procedure) until, after a minimum of two generations, stable inheritance is achieved and it is conclusively determined that the animals are not suffering any distress/discomfort as a result of the genetic changes in question. Other aspects of the new system can also affect the number of animals registered in the category of “died or killed before the procedure” versus “died or killed after the procedure”. The magnitude of these effects and their net result can only be assessed after a couple of years have passed. Data from the 2016 reporting year is expected to be sufficiently stable for the purposes of an initial evaluation of this kind.

4.1.4.2. Terminology used

The former CCD noted that the description “died or killed in stock” is not very applicable to the category of GM animals.¹⁸ After all, breeding programmes for GM lines often require complex crossing protocols, as a result of which not all of the progeny will have the genotype that is required for the purposes of the study in question. The remaining progeny will not possess the genetic modification (or modifications) and will usually be killed without ever having been used in a procedure. The research institutes report these animals under the categories “died or killed before use in breeding programme or animal procedure” or “died or killed after use in breeding programme” (NVWA Registration book). Until registration year 2013, “Zo doende” (the NVWA's annual review of animal procedures and

The freezing and thawing of embryos and semen should be routine practices to allow cryogenic storage of unique genetic material and strains that are (temporarily) not needed for animal experimentation

laboratory animals) reported these animals under the category of “died or killed in stock”. The reference to “stock” has a different connotation to “before use in breeding programme or animal procedure” or “after use in breeding programme.” Accordingly, the NCad recommends using the same description as the one given in the NVWA’s registration instructions.

More generally – in the interests of communicating with society – the NCad advises that “Zo doende” should be supplemented with guidance notes, explanatory notes, and an interpretation that are intelligible to the lay reader. The terminology used should be entirely unambiguous, i.e. there must be only one possible explanation for any given term.

4.2. Efficiency criteria

4.2.1. Generating GM lines

At the present time, only a limited number of academic centres in the Netherlands have laboratories that specialise in generating GM lines. These transgenic laboratories have been set up by scientists whose own research depends on the availability of specific GM lines. These scientists generate GM lines primarily for their own use. As a secondary consideration, they will also do so for fellow researchers with whom they are collaborating. Once these priority needs have been met, they will provide this service to third parties. In the context of inter-establishment cooperation on specific research themes, exchanges between fellow researchers are not restricted to the exchange of data, they also extend to the sharing of GM lines.

The best practice quality criteria in use today were drawn up on the basis of empirical evidence. These are the same criteria that the CAB originally used to establish the maximum number of animals (150) needed to generate a single GM line, using what are now seen as traditional transgenic methods.¹⁹ As previously stated, genome-editing technology is highly efficient. Partly for this reason, the NCad anticipates that it will be possible to reduce this number of animals substantially in the future.

4.2.2. Cryogenic storage

Any research institutions that breed GM animals must ensure that they have the infrastructure, knowledge and skills to deal with genetic material such as embryos and semen (germplasm). Unique genetic material and lines that, for the time being, are no longer needed for research purposes, can be cryogenically stored. However, this is only feasible if the freezing and thawing of embryos and semen have become routine procedures. Accordingly, it is important to use the same standard protocols wherever possible.²⁰ As a result, the exchange of GM lines (including those generated using genome-editing technology) can, in many cases, presumably remain limited to the exchange of embryos and/or semen. While prevention of the transport stress involved in exchanges of live animals needs to be taken into account, consideration must also be given to the impact of revitalisation on the animals involved and on the studies in question. Breeding coordinators and the IvD have a decisive part to play in such considerations. Furthermore, breeding coordinators and researchers must give due consideration to the use of biobanks as a source of quality-controlled (GM) strains, and when determining the need to

store embryos and/or semen from a given strain (or additional samples of such materials).

Those research centres that are not in a position to maintain such support infrastructure in-house should at least ensure that they have access to breeding programme support services provided by public institutions and consortia such as the European Mouse Mutant Archive (EMMA), which is now part of the above-mentioned INFRAFRONTIER.

4.2.3. CCD project licences: gender and age

The new gene-editing methods, such as CRISPR/Cas9, enable anyone with the right skill set and a knowledge of molecular biology techniques to create GM lines. This requires a project licence. This immediately provides points of reference for determining modified criteria, based on experience gained in practice, by the Central Authority for Scientific Procedures on Animals (CCD). Particularly with regard to the interpretation of ethical reviews, in which an animal's intrinsic value is the guiding principle, the CCD can supply specific guidelines for managing the numbers of animals killed in stock. When considering applications for a project licence, the CCD must routinely take into account whether the applicant in question has clearly shown that he/she has fully explored the aspect of animals that "died or were killed before being used in breeding programmes or animal procedures". This also applies to animals ordered from a laboratory animal supplier, e.g. by requesting a statement concerning the number of animals involved. This also applies to the IvD, in the context of the technical assessment of the proposed animal procedures under the project licence.

The NCad feels that applicants should provide comprehensive scientific justifications for their choice of inclusion criteria for animals for a given experiment, at least with regard to gender and to the maximum permissible age distribution. The guiding principle must be that, with regard to gender, both genders should be included in the procedure's design, and the age limits involved should not be unnecessarily restrictive. Opting to use animals of just one gender inevitably leads to a breeding surplus of the other gender. The selection of a limited age tolerance (for example, 6-week-old animals versus animals aged 6 to 8 weeks) inevitably leads to the need to breed more animals, in order to have sufficient breeding pairs that can simultaneously produce litters for the production of the required number of suitable progeny for the experiment.

4.3. Criteria and quality standards – Best practices

Genome-editing technology is increasingly replacing traditional methods that are technologically more complex, more labour intensive and more time consuming. The process can be further facilitated by asking those facilities that are pioneering the development and/or implementation of new genetic modification technologies to draw up additional criteria and quality standards for this area, pertaining to the generation and breeding of GM animals. Other, smaller, institutes must conform to these criteria and standards. They can also make their knowledge and expertise available to others, by providing training courses. This will facilitate rapid implementation and quality management across the various institutes (involving retrospective assessments to determine whether specific institutes conform to a particular standard). In addition, it

also makes sense for these specialised institutes to establish new criteria for the maximum number of animals needed to create a GM line.

The specialised institutes are also required to make statements (rather like those that are routinely issued in human healthcare) concerning the minimum frequency at which GM lines need to be generated in order to develop the skills required to generate and maintain such GM lines.

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Appendix 1 – Background information

Transgenic facility driven by science

The generation, breeding and use of GM animals for scientific purposes largely takes place at universities, university medical centres, and at the institutes of the Royal Netherlands Academy of Sciences (KNAW). In November 2014, at an initial meeting with representatives of “the largest users” of GM animals, the Minister for Agriculture asked them to make arrangements for putting into practice the recommendations of the international “Bred but not used” workshop and those of the former RODA. The representatives gave their views on the status of contemporary “transgenic practice” in the Netherlands. GM lines are generated in response to specific research objectives. Thus, creating GM animals is not an end in itself. At the present time, only a limited number of academic centres in the Netherlands have laboratories that specialise in generating GM animals. These transgenic laboratories have been set up by scientists whose own research depends on the availability of specific GM lines. These scientists generate GM animals primarily for their own use. As a secondary consideration, they will also do so for fellow researchers with whom they are collaborating. Once these priority needs have been met, they will provide this service to third parties. In the context of inter-establishment cooperation on specific research themes, exchanges between fellow researchers are not restricted to the exchange of data, they also extend to the sharing of GM lines. Those transgenic facilities that actually operate as facilities are managed by researchers who have research programmes of their own.

Only facilities such as these are capable of developing new technology in-house and/or of implementing technology developed by others.

Public licenced establishments should make due allowance for their public function. Accordingly, they should disclose information on any GM lines that have been generated (without breaching privacy), as well as intellectual property, by submitting these for registration in the pertinent databases.²¹ Sharing such information can help to ensure that the generation of a given GM line is not duplicated, even though genome-editing technology may make it simpler and more efficient to recreate a given line rather than to breed extra animals, or to store a line in the form of embryos and/or semen.

Creating GM animals

From 1 April 1997 to 1 January 2010, the creation of “transgenic animals” was subject to a “no-unless” policy. The creation of GM animals was prohibited, unless a licence for this purpose had been awarded by the minister.²² Licenses were awarded on condition that the procedures involved would have no unacceptable effects on the health and welfare of animals, and that there were no ethical objections to these procedures. To determine whether these conditions had been met, the Minister sought the advice of the Committee on Animal Biotechnology (CAB). On 1 January 2010, the Regulation regarding the exemption of biotechnological techniques for biomedical research entered into force. This caused the licensing requirement to lapse and, as a result, the assessment role was transferred to the Animal Ethics Committees (DECs).

The Committee on Animal Biotechnology took the view that a total of 50 animals would be needed to generate a transgenic or knockout animal (the “founder”) from which an entire GM line could be bred. Working on the assumption that three founders would be generated per line, the CAB arrived at a figure of 150 animals, as the maximum number needed to create a GM line.²³ The CAB’s advisory reports included details of which animals are involved in generating a GM line, and of applicants’ estimates concerning the degree of distress/discomfort that these animals might suffer.²⁴ In one of its advisory reports, the CAB expressed the following view: In a well-equipped transgenic laboratory with experienced staff, an average of 150 animals should be sufficient to generate a GM mouse line, through the microinjection of embryonic stem (ES) cells into blastocysts.²⁵ In formulating its advisory report, the CAB took account of the state-of-the-art (in terms of the technology involved) and of the “quality” of the applicant.

Technological developments and their expected impact

The ISTT is an international organisation with global operations in the field of the transgenesis of animals used for scientific purposes.²⁶ The organisation’s 3R Committee has shared its views on the effects of using gene-editing methods such as CRISPR/Cas9.^{27, 28} In brief, this states that the technology has been in use for several years now, that it is very promising, and that the specificity with which changes can be made will improve as the technology is refined still further. The 3R Committee anticipates that there will be a decline in the number of animals needed to create a transgenic line. In addition, the technology has the potential to create several modifications at once.

This would result in fewer animals being bred in cases where, previously, it had been necessary to cross GM animals, each with different single modifications, to achieve the desired combination of single modifications in a single animal. The 3R Committee anticipates that it will take up to 10 years to reach the required level of specificity and efficiency. In addition, traditional genetic modification technology is unable to create GM animals in species for which no embryonic stem cells are available. However, the ISTT Committee notes that gene-editing technology does indeed hold out the prospect of creating GM animals using these species. The first GM rats, goats, and Marmoset monkeys have already been created. Breeding programmes will be established for these newly created GM lines, after which they will contribute to the total number of animal procedures and to the total number of animals killed before the procedure. However, the ISTT’s 3R Committee anticipates that the use of CRISPR/Cas9 gene-editing technology will not significantly boost the number of laboratory animals. The Committee takes the view that, over the long term, there will be opportunities to reduce the number of animals that are killed in stock.

Others expect a marked increase in the number of GM animals used, due to the potential for new disease models in higher species and to the successive maintenance of an increasing number of GM-animal lines. They conclude that this goes against current policy in the Netherlands and the European Union (Hendriksen & Spielmann, 2014).

Various international experts in genetic modification have added some remarks of their own to the ISTT’s 3R Committee’s conclusions.

The development of gene-editing technology and its applications is growing exponentially. Accordingly, it is not realistic to define a timeframe. In the context of this request for advice, the most relevant remark concerns the technology's impact on breeding programmes for GM animals. With just a single genome-editing experiment, laboratories that use CRISPR/Cas9 technology can generate dozens of potentially interesting mutant lines, compared to just a few using traditional transgenic methods. At the present time, breeding programmes to derive stable lines from these lines constitute animal procedures. Following selection on the basis of biological relevance, if the remaining lines include several stable lines that are relevant to the study in question, subsequent breeding programmes for these lines can result in an increase in the number of animals in the category "died or killed in stock". However, it is realistic to assume that more scientific knowledge will be obtained from each individual animal than was previously the case.

Conditioned GM models

Pursuant to the recommendations of the above-mentioned 2013 international workshop and those made by RODA, current breeding practices have been discussed in consultations with the largest users, and remarks have been appended. Here, it was concluded that there has been an increase in the breeding of conditioned GM models. Conditioned models involve genetic modifications that are only expressed once they have been switched on, and then often only in the specific tissue or organ that is being investigated; these are refinements of models in which the modified genes were permanently switched on throughout the entire organism, or deleted

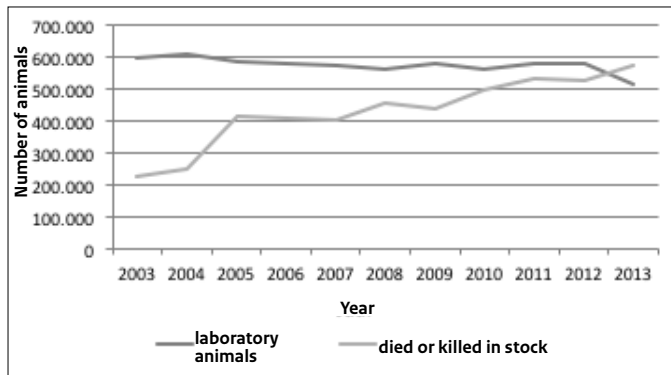
from all of the organism's tissues (knock-out animals). Conditioned models are more biologically relevant as the genetic change can be specifically switched on in the tissue or cell type in which the gene in question is normally expressed, and at a time that is relevant to the study of a given disease model. There are various ways of switching genes on or off. However, the method that involves crossing a line with a second, different GM line that provides the signals required to switch on the genetic modification in the first line is of particular relevance in the context of the number of animals that are killed before the procedure. After all, in this case, a single conditioned model requires breeding programmes to be established for several GM lines, all of which can potentially contribute to the number of animals that are killed before the procedure. The advantage of these "conditioned models" is that, until the genetic change is switched on, the animals experience no adverse effects from this change with respect to their physical characteristics. Accordingly, there is less risk of welfare compromises. Ultimately, it is a question of whether the greater number of animals that need to be bred for a given model outweighs the increased biological relevance of that model and the associated reduction in welfare compromises.

NVWA - annual registration

Each year, in accordance with the Wod, the establishment licensees report details of the number of animal procedures and laboratory animals to the NVWA (the Netherlands Food and Consumer Product Safety Authority; <http://www.nvwa.nl>). The NVWA publishes the registered data in "Zo doende", its annual review. The NVWA reports details of the animals that were bred but not used in experiments

under the heading “died or killed in stock”. This is a collective term for animals that, for various reasons, were not used in experiments.

In 2013, the number of animals that were killed in stock exceeded the number of animals that were actually used in experiments.



Total number of animals in the category “died or killed in stock” and the number of laboratory animals. Source: “Zo doende” (the NVWA’s annual review of animal procedures and laboratory animals), 2003-2013

In response to the numbers of animals reported in the category of “died or killed in stock” in 2011, the NVWA conducted a survey of licenced establishments that are licenced to breed laboratory animals. The aim of this survey was to better understand the category of “died or killed in stock”.²⁹ The results of the survey were as follows:

1. a maximum of 5% can be attributed to disease or other health problems;
2. approximately 13% are ex-breeding animals that are not suitable for experimental purposes;
3. approximately 22% are unsuitable due to their age;
4. approximately 23% are of the “wrong” gender; and
5. approximately 38% do not have the desired genetic composition (“unsuitable genotype”).

At European level, a European Commission Expert Working Group (EWG) tasked with statistical reporting published a working document in January 2012. This document addressed the matter of how, in general terms, the generation, breeding and use of genetically modified animals might be authorised and how this should then be statistically reported by the Member States of the European Union. This working document should be seen as a guideline. The Dutch government has endorsed this guideline’s recommendations and conclusions.

Given that, under the revised Dutch Experiments on Animals Act, animal procedures may only be carried out by holders of project licences, such a license is required for:

- generating a GM animal until the line can be considered as “established” or stable, i.e. when the genetic change or changes in question have been stably inherited for at least two generations, and an estimate has been made of the anticipated level of distress/discomfort. The appendix to the EWG’s working document specifies the key elements of a welfare assessment scheme.

- The breeding of GM animals that have an adverse phenotype.
- The breeding of GM animals that are at risk of developing an adverse phenotype, such as a disease or a tumour, as well as GM animals whose immune system is compromised and who are, therefore, at risk of infection. This is regardless of the measures taken, including sacrificing the animal before the adverse phenotype has manifested.

It may be some time before it is discovered that, in spite of previous evaluations, a GM line has a welfare compromise that must be attributed to the genetic change in question. If that is indeed the case, then an adverse phenotype is involved and a project licence will be required to establish a breeding programme for the GM line in question.

A project licence is not required for GM lines that require a specific, deliberate action to actually make the genetic modification or modifications in question biologically active. These lines are known as induced GM lines.

Registration for the Dutch authorities has been adapted such that any parental animals that are killed or are found to have died, and which have not been used in an experiment, must be registered as a separate category. This also applies to animals that are killed or are found to have died before being used in an experiment or breeding programme, for reasons of unsuitability or redundancy. Under the new registration system, too, animals are only included once they have been weaned but also when they are used in a procedure before weaning; none of the foetal stages during the last third of the

gestation period will be recorded, unless such animals were used in the procedure and were born alive or in a viable state during the procedure. In rats, for example, this corresponds to day 20 of gestation.

Appendix 2 - recommendations arising from the consultation of community groups

Breeding coordinators

Netherlands Federation of University Medical Centres (NFU)

When designating a breeding coordinator, it is important not to underestimate the complexity of breeding programmes. The way in which this is implemented must be in keeping with the size and complexity of the colony. The NFU representative explains this in greater detail.

An effective information system (with IT support, in complex situations) is essential to generate relevant data in real time, to monitor assignments, to see what happens, and to be able to use short-cycling interventions.

Taken into account in the advisory report: yes

Three Rs Alternatives Initiating Network (TRAIN)

The appointment of a breeding coordinator is highly desirable, partly because of the positive experiences to be gained. That individual will then specifically monitor the supply and demand of laboratory animals within the licenced establishment. However, a role of this kind could also be assigned to the IvD.

The research community has indicated that it is already using such individuals in this role. Yet this is seen as a valuable investment, one which holds out the option of peer review.

It is not a good idea to impose things from above.

Based on past experience, the coordination of breeding programmes could be carried out by two individuals. One would be an animal welfare officer, the other an individual who is closely associated with ordinary workers, as single individuals seldom have the requisite breadth of knowledge.

Taken into account in the advisory report: yes

Royal Netherlands Academy of Sciences (KNAW)

It is useful to appoint a breeding coordinator. Furthermore, this individual should be given the necessary resources, such as the right tools for retrospectively modifying breeding programmes. However, this method has not yet been widely accepted and introduced.

Taken into account in the advisory report: yes

Netherlands Association for Laboratory Animal Science (NVP)

With regard to the effective planning of breeding programmes, the NVP representative feels that one group has been omitted, namely researchers; Planning is not their core business and some things, by their very nature, cannot be planned anyway. Nevertheless, this group should be involved in carefully planned breeding programmes.

Breeding coordinators might be able to play a useful part in this, assessing the level of coordination by transparent, analytical means.

The NVP has reservations about appointing a breeding coordinator, however, as questions have been raised about whether this individual

would have a sufficiently strong mandate to exercise any influence. Taken into account in the advisory report: No, the appointment of a breeding coordinator will not be recommended. The NCad is unaware of any factors that might impede the mandate.

Vision regarding the modification of breeding practices/ vision of centralisation

Netherlands Federation of University Medical Centres (NFU)

There is little scientific support for physically centralising live animals. As a result, the individual end users obtain their animals from different sources. The objections to this situation include differences in microbiological quality and the potential stress effects of transport.

Anyone can acquire GMO lines simply by purchasing various existing lines and crossing them with one another.

Yet only a few institutes currently generate their own lines. Even fewer laboratories have the skills needed to routinely carry out modifications in embryos. Carrying out such work on a sporadic basis results in inherently lower efficiency levels (as more animals are required). The best way to guarantee the quality of transgenic procedures may be to assess routine and efficiency, as the Dutch health service does.

Taken into account in the advisory report: yes

The NFU representative expressed no preference on the matter of whether or not such techniques should be embedded in legislation or whether it is sufficient simply to use performance indicators (including outcomes).

Royal Netherlands Academy of Sciences (KNAW)

The KNAW's vision is to be very cautious when using policy-based measures to manage the centralisation of breeding programmes. Giving the incredibly fast pace of developments, in just a few years virtually any institute will be able to apply techniques that, at the moment, can only be carried out by specialists. If breeding programmes were to be centralised at specialised institutes, researchers would probably still be confronted by issues such as transport and, possibly, import. In addition, any infections at centralised breeding establishments would have a much greater impact on the animals and studies involved. Moreover, it is questionable whether this would reduce the efficiency of breeding programmes by reducing the magnitude of breeding surpluses. Indeed, the possibility cannot be excluded that this might even increase. Accordingly, the KNAW's representative opposes the imposition of centralisation.

Taken into account in the advisory report: yes

Professional group of animal welfare officers

It is highly desirable for a standard of decency, in some form, to be observed in the field of animal breeding. This is gradually beginning to emerge in dealings between companies and their clients, in terms of better arrangements about the number of animals being bred. It is clearly desirable that such a standard should also be introduced into dealings between licenced university establishments. A step of this kind could be initiated by the government. However, this is not achievable in the short term as it involves an international network of licenced establishments, animal breeders and other companies.

Taken into account in the advisory report: no, this is a statement

Netherlands Association for Laboratory Animal Science (NVP)

While it is not desirable to create centres of excellence, guidelines should nevertheless be drawn up to indicate how breeding lines should be generated and managed. As in the Dutch health service, quality should be monitored by indicating a minimum frequency for the performance of a particular technique. No statements are made as to whether such guidelines should be imposed from above. However, these guidelines are important for the NVWA, in terms of its enforcement work.

The NCad should draw up a guideline, to include details of breeding colony management and a role for breeding coordinators. In this connection, a role is also envisioned for the IvD. This would involve monitoring efficiency within a licenced establishment. The Netherlands is still too fragmented. There are, of course, various practical reasons for this, some of which are infrastructure-related. As a result, however, plans that involve several different licenced establishments are not optimally coordinated. This, in turn, means that exchanges of animals are not really feasible. One result of this is that there are more breeding surpluses at local level. Accordingly, the NVP is pushing for better coordination between licenced establishments.

Taken into account in the advisory report: yes, in part. It is recommended that research groups pioneering the development and/or implementation of new genetic modification technologies be closely involved in drafting guidelines, to ensure the support of the research community. These groups can then operate as quality ambassadors, training others and encouraging them to phase-out obsolete technologies.

Wil Research (on behalf of the industry)

The IvD can play a pivotal role helping licenced establishments to adopt proper breeding practices, as it has a mandate to check whether licenced establishments are using state-of-the-art techniques, and whether the appropriate knowledge and experience is available. If this is linked to codes of best practice and clear co-ordination, a great deal can be achieved with respect to the efficient breeding of transgenic lines. This sort of approach can be incorporated into any kind of structure, enabling centralisation to be achieved from the bottom up. If this were to be regulated, it would result in rising costs and diminishing flexibility. That would be a most unwelcome development. Taken into account in the advisory report: yes, in part.

HollandBio

The IvD could play a part by helping to make technological developments within licenced establishments more transparent. It is more desirable to show how the number of animal procedures was arrived at, rather than focusing purely on the number of animal procedures carried out. While these numbers are societally relevant, it is important to clearly indicate the amount of knowledge that they have generated. In terms of knowledge, the Netherlands has generated a leading position in the world and this needs to be maintained.

Taken into account in the advisory report: no, since this point is already addressed in the advisory report: “INDICATORS, MANAGEMENT AND UTILISATION OF DATA FOR MONITORING LABORATORY ANIMAL USE AND 3R ALTERNATIVES”.

Impact of legislation/registration

Netherlands Federation of University Medical Centres (NFU)

The NFU representative refers to the modification of the registration system, and explains what this involves. The registration system in the Netherlands has already been modified to make it possible to distinguish between animals that are killed after breeding versus those that are killed before being put to any use at all. This can help to clarify matters.

Furthermore, the number of animal procedures can be expected to increase, even if the work programme remains at the same level of intensity. That's partly because European regulations require that, for all new modifications (or combinations thereof), a minimum number of progeny should be carefully monitored and registered as animal procedures. This requirement applies regardless of the likelihood of additional distress/discomfort. The effect of the change in the registration system (resulting from changes in legislation and regulations) will not be fully visible until 2017 (some effects will only appear gradually, also the pan-European statistics will not be published until 2017).

Taken into account in the advisory report: yes

Three R's Alternatives Initiating Network (TRAIN)

The TRAIN representative drew attention to the category of animals which are not transgenic but which are killed. To get a clear understanding, it is important to sub-divide the advisory report and make it transparent.

Taken into account in the advisory report: no, in accordance with the Minister for Agriculture's request, the advisory report is limited to transgenic animals, and, more particularly, to mice and fish.

Professional group of animal welfare officers

It is understandable that the advisory report emphasises genetically modified animals, but it does not lose sight of non-genetically modified animals. Society draws no distinction in this regard, and the non-GM breeding group also includes surplus animals. This is why it was felt to be desirable for non-genetically modified animals to be included in the advisory report. There is no immediate concern about policy regarding the breeding of genetically modified animals. The only problem is its lack of transparency, which is neither necessary nor – in the long term – advisable.

Taken into account in the advisory report: no, in accordance with the Minister for Agriculture's request, the advisory report is limited to transgenic animals, and, more particularly, to mice and fish.

Impact of technological developments. **Fish/mice**

Netherlands Federation of University Medical Centres (NFU)

With regard to the sacrifice of animals, there is no objection to reviewing fish separately. This would initially involve establishing contacts between individual laboratories, and submitting some inventory questions to them. Any remaining issues can be examined at a later stage. It is often the case that researchers only use embryos, so the adult animals required usually fall outside the definition of an animal procedure.

Taken into account in the advisory report: no, this is a statement

CRIPR/Cas9 is a promising technology that is not restricted to a single species, the mouse. It can be used more generally, for other species as well. The technique is more efficient than traditional techniques. It is not easy to predict what direction this application will take in other species. It requires the proper infrastructure (such as the technology needed to handle embryos) and the laboratory facilities required to use this technique technology effectively (e.g. verification of the change in the genome). Taken into account in the advisory report: yes

Three R's Alternatives Initiating Network (TRAIN)

It is anticipated that the new CRISPR/Cas9 technology will be rolled out in every institute, as the traditional method of producing transgenes is much more expensive. This can be facilitated by making the knowledge and expertise involved readily available and by providing training courses. An approach of this kind will greatly accelerate implementation throughout the various institutes. The research community that works with fish is a very open community indeed. There is no such openness in the research community that works with mice. However, if the present rapid pace of technological development is maintained, it is anticipated that a similar degree of openness will develop in the latter research community. Taken into account in the advisory report: yes

Royal Netherlands Academy of Sciences (KNAW)

Ideally, these new technological developments should not be strictly regulated, nevertheless it is important to keep a finger on the pulse.

It is a simple matter to produce transgenes in fish, and development in this area has been very rapid indeed.

In the breeding of transgenic mice, too, the pace of technological development is tremendous.

Taken into account in the advisory report: yes

Cryopreservation

Netherlands Federation of University Medical Centres (NFU)

Cryopreservation is becoming increasingly accessible. If colony management is organised effectively, a new technology of this kind can deliver gains in terms of reducing the number of animals killed in stock.

If researchers start importing embryos and semen, then qualified centres must be willing to share their knowledge, skills and routines to revitalise lines for third parties as well.

After pointing out that it is not possible to switch entirely to cryopreservation, as the Max Planck Institute has done (example given), the NFU representative explained why this is (the potential increase in the number of animals needed for revitalisation).

Taken into account in the advisory report: yes

Three Rs Alternatives Initiating Network (TRAIN)

The Max Planck Institute (which has a large number of freezers) was mentioned in the context of discussions about cryopreservation. It might be useful to consult this institute, to find out what this development might mean for future investments in the Netherlands,

in terms of deep-frozen material versus living material. However, it would be advisable to include an economic analysis in that context.

Taken into account in the advisory report: yes

Netherlands Association for Laboratory Animal Science (NVP)

From a purely financial perspective, researchers often prefer to continue using a couple of breeding pairs for a few years instead of getting involved in cryopreservation. Cryopreservation may, however, ultimately reduce the number of animals killed in stock. Accordingly, this method should be included in the advisory report.

Taken into account in the advisory report: yes

Other topics

Exchanging lines

Royal Netherlands Academy of Sciences (KNAW)

Institutes want/are required to maintain an in-house Specific Pathogen-Free (SPF) status (reproducibility of results). This poses a huge barrier to the free transport of transgenic mice between different research institutions (both nationally and internationally). The associated rederivation procedures (to remove adventitious organisms) result in additional animal procedures (and in the loss of a considerable amount of time for the study).
This is a statement

Generating new lines

Royal Netherlands Academy of Sciences (KNAW)

It is advisable to ask some of the specialised institutes that are leading the way in the development of new technology for the creation of new genotypes to draw up a type of quality standard with which other, smaller, institutes must comply. This will facilitate quality management, involving retrospective assessments to determine whether specific institutes meet a particular standard.

One such quality standard, for example, relates to the maximum number of animals to be used in creating a new line.
As yet, there are no such criteria for modern technology.

According to the former Biotechnology with Animals Decree, no more than 150 animals are needed to produce a transgenic line. However, the new technologies may make it possible to reduce this number. Thus, it is recommended that further research be carried out to determine how many animals are actually required to create a new genotype, using the new technologies.

It is not a good idea to impose strict standards and criteria from above.

The rapid development of technology threatens to exacerbate the conflict between not being permitted to create a new line if it already exists and importing such a line yourself (involving, in most cases, rederivation or embryo transfer). Both courses of action also involve the use of animals, and sometimes considerable costs (the rederivation of a line can cost up to €4000).

Taken into account in the advisory report: yes

Footnote

- 1 International workshop “Gefokt maar niet gebruikt” (“Bred but not used”); Appendix 3 to the letter from the Minister for Agriculture to the President of the House of Representatives, dated 28 February 2014, reference DGA-DAD / 13205800, concerning: Action Plan for Animal Procedures and Alternatives.
- 2 Guidance letter from the RODA to the Minister for Agriculture, entitled “Animals sacrificed in stock”, dated 25 September 2014.
- 3 Until the registration year 2013, in accordance with the Dutch Experiments on Animals Act (Wod), establishment licensees registered these animals in the category “sacrificed or died before the procedure”. In “Zo doende”, its annual review of animal procedures and laboratory animals, the Netherlands Food and Consumer Product Safety Authority (NVWA) reported these animals under the heading “died or sacrificed in stock”. In 2014, the categories “died or killed before use in breeding programme or animal procedure” and “died or killed after use in breeding programme” were added to the notes to the registration of laboratory animals and animal procedures.
- 4 Morality – a coherent set of beliefs that an individual or group use as a guideline to leading a good life (individually and collectively) or to doing the right thing. Ethics critically and systematically examines and questions morality.
- 5 In the registration year 2013, the NVWA reported the following in its annual review “Zo doende”: 574,511 animals were categorised as “died or killed in stock”, 446,026 (78%) of which were GM animals.
- 6 Letter from the Minister for Agriculture to the chairman of the Netherlands National Committee for the protection of animals used for scientific purposes, dated 31 March 2015, reference DGA-DAD/15040736, concerning: Request for advice on the management and utilisation of data on animal procedures and alternatives, animals sacrificed in stock, rehoming scheme for former laboratory animals, pain management and procedures involving cats and dogs.
- 7 Letter from the chairman of the Regular Consultation Body on Animal Procedures and Alternatives (RODA) to the Minister for Agriculture, dated 25 September 2014.
- 8 International workshop “Gefokt maar niet gebruikt” (“Bred but not used”); Appendix 3 to the letter from the Minister for Agriculture to the President of the House of Representatives, dated 28 February 2014, reference DGA-DAD / 13205800, concerning: Action Plan for Animal Procedures and Alternatives.
- 9 Letter from the Central Authority for Scientific Procedures on Animals to the Minister of Health, Welfare and Sport, “Request for advice regarding the centralised breeding of transgenic animals”, dated 29 April 2011
- 10 The “breeding coordinators’ consultation” platform is currently being established, under the aegis of the Biotechnical Association. The first meeting took place on 22 June 2015.
- 11 Letter from the Central Authority for Scientific Procedures on Animals to the Minister of Health, Welfare and Sport, “Request for advice regarding the centralised breeding of transgenic animals”, dated 29 April 2011
- 12 The “breeding coordinators’ consultation” platform is currently being established, it operates under the aegis of the Biotechnical Association. The first meeting took place on 22 June 2015.
- 13 “Forward genetics” is an application in which the genetic basis of an abnormal phenotype is determined. In this approach, male animals are treated with chemicals or radiation, to generate random mutations in sperm cells. The mutation-carrying progeny of these males are examined for the presence of an abnormal phenotype. The next step is to identify the responsible gene or genes.
- 14 Reverse genetics is an approach that investigates the function of a gene, based on an analysis of the phenotypic effects of specific modified genes.
- 15 <http://www.infrafrontier.eu>
- 16 European Directive 2010/63/EU Article 27 paragraph 1.
- 17 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 in stock after the procedure”; with effect from the registration year 2014, 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 of the procedure”, “after use in the procedure”. Sources: Explanatory notes to the 2013 and 2014 registration of laboratory animals and animal procedures, by the Netherlands Food and Consumer Product Safety Authority (NVWA).

- 18 Letter from the Central Authority for Scientific Procedures on Animals to the Minister of Health, Welfare and Sport, "Request for advice regarding the centralised breeding of transgenic animals", dated 29 April 2011.
- 19 Letter from the Committee on Animal Biotechnology to the Director of Veterinary, Food and Environmental Affairs, Ministry of Agriculture, Nature Management and Fisheries, reference CBD/01.54/RT, 8 March 2001.
- 20 <https://www.infracfrontier.eu/knowledgebase/protocols/cryopreservation-protocols>.
- 21 IMSR – International Mouse Strain Resource (<http://www.findmice.org>); MGI - Mouse Genome Informatics (<http://www.informatics.jax.org>); RGD – Rat Genome Database (<http://rgd.mcw.edu>); RRRC - Rat Resouce & Research Center (<http://www.rrrc.us>); ZFIN – The Zebrafish Model Organism Database (<http://www.zfin.org>).
- 22 Animal Health and Welfare Act (GWWD), Article 66, paragraph 1.
- 23 Letter from the Committee on Animal Biotechnology to the Director of Veterinary, Food and Environmental Affairs, Ministry of Agriculture, Nature Management and Fisheries, reference CBD/01.54/RT, 8 March 2001.
- 24 Distress/Discomfort: causing pain, suffering, distress or lasting harm.
- 25 <http://www.rijksoverheid.nl/documenten-en-publicaties/rapporten/2007/10/26/besluit-biotechnologie-bij-dieren-advies-cbd-over-rbdo203-105.html>
- 26 International Society for Transgenic Technologies; <http://www.transtechsociety.org>.
- 27 Towards the 3Rs of laboratory animal science: Replacement, Reduction and Refinement (Russell WMS, Burch RL (1959) The principles of humane experimental techniques. Methuen and Company, London) Towards the 3Rs of laboratory animal science: Replacement, Reduction and Refinement (ibid.).
- 28 Jerchow, B. 2014. Statement by the ISTT's 3R Committee.
- 29 NVWA report entitled "Died in stock", 17 December 2013; Appendix 1 to the letter from the Minister for Agriculture to the President of the House of Representatives, dated 28 February 2014, reference DGA-DAD / 13205800, concerning: Action Plan for Animal Procedures and Alternatives.
- 30 National competent authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes – Corrigendum of 24 January 2013 – Working document on genetically altered animals. Brussels, 23-24 January 2013. http://ec.europa.eu/environment/chemicals/lab_animals/pdf/corrigendum.pdf
- 31 Explanatory notes to the Netherlands Food and Consumer Product Safety Authority's 2014 Registration of Laboratory Animals, December 2014.

Procedure

This advisory report is based on the recommendations that emerged from various consultations, initiated by the Minister for Agriculture, amongst others.

One of these consultations was the 2013 international expert workshop “Bred but not used”. The recommendations that emerged from this workshop were submitted to the former Regular Consultation Body on Animal Procedures and Alternatives (RODA), which was asked to work out the details. The recommendations of the former RODA have been incorporated into the progress report for the “Action Plan for Animal Procedures and Alternatives”, which was presented to the House of Representatives in November 2014. This document contained an announcement about a consultation with the largest users, which took place in November 2014. Various recommendations that emerged from this consultation with the largest users (and the one that followed) and with the Netherlands Food and Consumer Product Safety Authority (NVWA), as well as from consultations with international experts, are included in this advisory report. Also included, is the input from the public consultation that was held on 5 June 2015.

Due to personal circumstances, one of the members of the NCad was neither able to take part in the final discussions nor in completing the advisory report. There is full consensus among the remaining members with regard to the content of the advisory report.





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The Netherlands National Committee for the protection of animals used for scientific purposes (NCad) was appointed for the protection of animals used for scientific purposes and for education. The NCad achieves visible improvements that are specifically related to the Replacement, Reduction and Refinement (3Rs) of animal procedures and to the associated ethical review in scientific research (including applied scientific research) and teaching. Its goal, in doing so, is to minimize laboratory animal use at both national and international level.