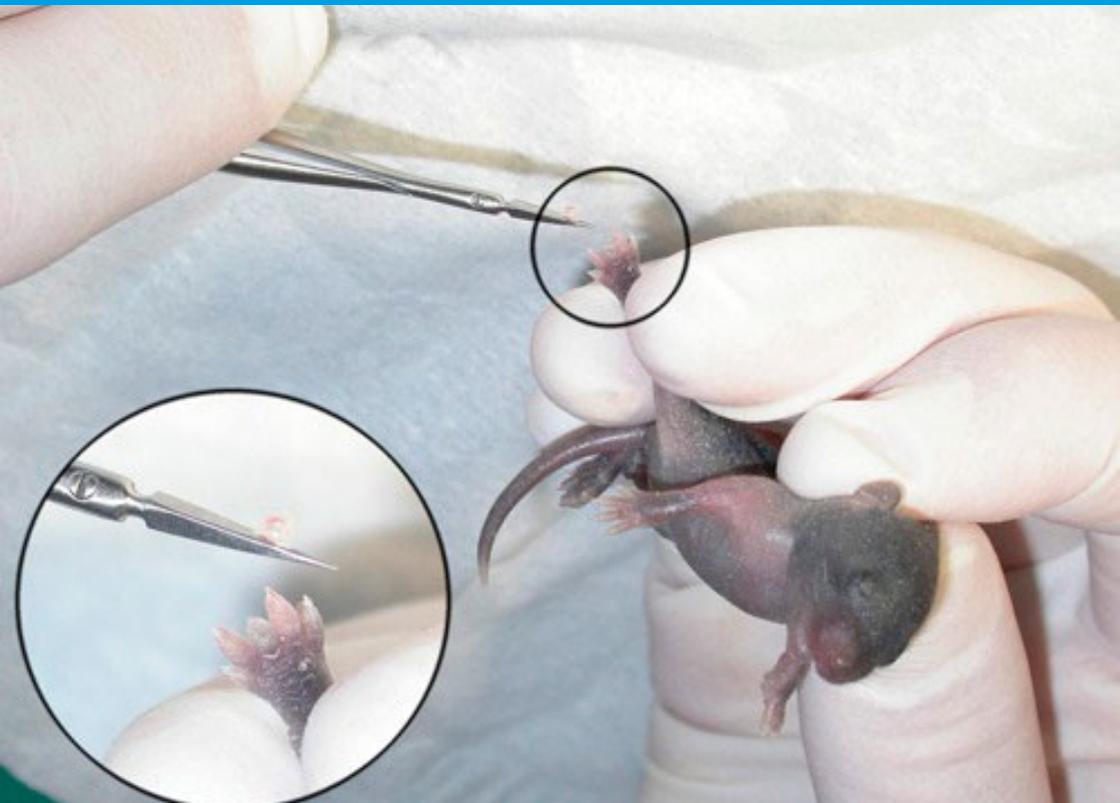




Netherlands National Committee
for the protection of animals
used for scientific purposes

Careful assessment of methods for identification and genetic characterization



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.

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*Cover image:
Restriction and cutting of the distal phalanx
of seven day old mouse pup*

Image of Dagmar Schaefer, Zürich

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Summary

In October 2018, the Minister of Agriculture, Nature and Food Quality asked the NCad to publish an advisory report concerning the practice of toe clipping, based on previous research findings and existing guidelines.

Both toe/distal phalanx clipping and ear notching are used for the identification and genetic characterisation of genetically modified (GM) mice. These procedures are preferably conducted well before weaning age to enable genotyping results to be collected prior to weaning age.

The same arguments are often used to disqualify the use of one method in favour of the other, such as the degree of discomfort or stress, the impact of mutilating a body part and the age at which the procedure is performed.

In practice, due to the extremely small size of the pups at the moment the phalanx is biopsied, the attempts to only clip the distal phalanx result in the majority of the toe being removed. Therefore, this procedure is hereinafter referred to as ‘toe clipping’.

Other methods of characterising animals include collection of droppings, sampling of oral mucosa, aqueous humour, rectal swabs or the plucking of hairs with the follicle attached. Animal identification can be done by shearing fur, applying paint marking, metal or plastic earmarking, or subcutaneous ink marking, tattooing, implanting subcutaneous chips, or accommodating the animals individually.

Characterisation and/or identification methods can be classified in accordance with three criteria:

- invasive or non-invasive;
- identification only, genetic characterisation only or a combined procedure;
- temporary or permanent identification mark.

Genetic characterisation (genotyping) is not necessary for non-genetically modified animals. In such cases, identification of individual animals alone is sufficient for the purposes of an experiment.

All current temporary identification methods are non-invasive, and all current permanent identification methods are invasive.

Currently, no non-invasive methods are available that will simultaneously characterise and identify the animals via a single procedure, although it is possible to combine non-invasive characterisation methods and non-invasive identification methods. Combined procedures require more frequent handling and restraint of every individual animal, and the restraint of animals in particular causes a great deal of stress. Such combined procedures must be designated as experiments on animals if they impair the welfare of the animals to or beyond the extent of the lower limit stipulated by law. These combined procedures can be carried

out either before or after the weaning age in accordance with best practices. Contradictory information has been published concerning the impact on the animal during the conduct of various types of characterisation and identification. Furthermore, no effective indicators are available to estimate the degree of impact that toe clipping and ear notching have on animal welfare. Evidence that these methods impair the animal's welfare does exist, although none exists to show whether one method causes greater impairment than the other. Based on the aforementioned information, it is not possible to establish a sufficient scientific argument to either ban the practice of toe clipping and/or ear notching or permit both. However, the NCad considers it plausible that a combination of non-invasive methods will impair the animal's welfare to a lesser extent than the invasive methods. In every applicable situation, the parties involved have a duty to meticulously assess what the most suitable identification and characterisation methods would be in view of aspects such as the impairment of integrity, the impairment of welfare and the improvement of the quality of science.

It is recommended that the Ministry of Agriculture, Nature and Food Quality as well as the Ministry of Education, Culture and Science initiate a specific project aimed at developing combinations of sustainable, non-invasive identification and characterisation methods, in which impairment of the integrity and welfare of the animal is eliminated or minimised. In addition, further research must be conducted into innovative identification and characterisation methods for laboratory animals, such as individual facial recognition and DNA isolation/analysis techniques. Finally, the evaluation of the Experiments on Animals Act (*Wet op de dierproeven*, Wod) must incorporate the effect on laboratory animal welfare and integrity, of procedures that are not covered by the definition of 'experiment on animals' but are closely related to it.

Background

In October 2018, the NCad was asked by the Minister of Agriculture, Nature and Food Quality to publish an advisory report concerning the practice of toe clipping on rodents. This letter stated that the Dutch Lower House had requested¹ that the government phase out the use of toe clipping as a method of identifying laboratory animals. The Minister responded that she would be willing to ban toe clipping provided a suitable alternative method was available.

The NCad was also asked to investigate the extent to which other EU Member States use alternative methods of genetic characterisation² (genotyping) and identification of individual rodents. Furthermore, the NCad was asked to use the findings from this investigation to formulate a Code of Practice. The Ministry of Agriculture, Nature and Food Quality stated that the basic principle is that no unnecessary pain or injury to animals may be caused³ and that any methods causing such pain or injury may not be performed in the event that a 3R alternative is available.

Meticulous assessment must be conducted to determine which identification and/or genotyping methods are absolutely necessary for specific types of study and result in the least amount of stress for the animal.

Subsequently, the procedures carried out on the animal must be conducted in as responsible a manner as possible. The Ministry states that the NCad can use previous research results^{13,14} and existing guidelines⁴ as a point of departure for the research.

¹ Amended motion submitted by member Ouwehand (for the benefit of Parliamentary Paper 33692, No. 36) on phasing out toe clipping;

² Definition: the taking of a sample that is suitable for carrying out genetic characterisation.

³ Article 2(6) of the Disease Specialists Decree (*Besluit Diergeneeskundigen*).

⁴ Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification; *Laboratory Animals* 2013; 47: 2-11. DOI: 10.1177/002367712473290.

Introduction

Toe/distal-phalanx clipping is one of the methods used for the identification and genetic characterisation of genetically modified (GM) mice.

Genetic characterisation (genotyping) is not necessary for non-genetically modified animals. In such cases, identification of individual animals alone is sufficient for the purposes of experiment.

GM mice are bred with the aim of creating mice with the desired genetic modification. According to Mendel's laws of inheritance, only a portion of the pups bred inherit the parents' modified genes. To determine which pups are carriers of the desired modified genes, a tissue sample is taken at a young age from which DNA is isolated and analysed to determine the presence or absence of the genetic modification. This process is known as genetic characterisation or genotyping. In practice, characterisation is often combined with identification by means of the toe or ear tissue that is removed for the purposes of the characterisation. The tissue samples are taken well ahead of the pups' weaning age (21 days). This does not constitute an animal procedure in the eyes of the law.⁵ Animals with a DNA profile that is unsuitable for the experiment or for breeding will subsequently be killed prior to the weaning age and therefore do not count towards the statistics in the laboratory animal register.

In order to link the results of the genetic characterisation to the correct animal, the tissue sample must be accompanied by a unique identification marker. This unique identification marker must remain recognisable for at least as long as there is a realistic chance that the animal in question could be confused with animals of a different genotype. Among other factors, this depends on the structure of the experiments for which the animals have been bred and the requirements placed on the animals in relation to the necessary genotypes, sex and age of the animals at the time of the experiment. The current practice for the simultaneous identification and characterisation of GM animals is to take a sample from the ear (ear notching) or the toe (distal phalanx clipping).

In 2013, the Federation for European Laboratory Animal Science Associations (FELASA) established guidelines for the refinement of characterisation methods for GM mice⁴. The guidelines recommend distal phalanx biopsy,⁶ a practice that involves the removal of the distal phalanx of a maximum of two toes on different paws, rather than toe clipping.

⁵ Article 1b(7)(e) of the Experiments on Animals Act (*Wet op de Dierproeven*, Wod) states that "this act does not apply to practices whose main purpose is the identification of animals".

⁶ Only remove the distal phalanx from one toe on a maximum of two paws of pups aged seven days.

However, in practice, due to the extremely small size of the pups at the moment the phalanx is clipped, the attempts to only clip the distal phalanx result in the majority of the toe being removed. Therefore, this procedure is hereinafter referred to as ‘toe clipping’.

The practice of ear notching involves the clipping or punching of one or more triangles or circles from one or both ears. As with toe clipping, this method should preferably be conducted well ahead of the weaning age to enable genotyping results to be collected prior to weaning age.

In addition, other procedures for the genetic characterisation of animals exist that do not simultaneously leave a unique mark on the animal in question to enable subsequent identification. However, it is futile to take a sample from an animal for the purposes of genetic characterisation unless the results can be indisputably traced back to the animal in question. For this reason, it only makes sense to perform genetic characterisation if the sampling method leaves an identification mark on the animal.

Various methods are available to mark animals for identification purposes. An extensive description of identification methods can be found in the aforementioned FELASA guidelines.

The characterisation and/or identification methods can be classified into the following categories:

- Invasive or non-invasive (does it involve penetration of the body or not?);
- Identification only, genetic characterisation only or a combined procedure;
- Temporary or permanent identification mark.

All current temporary identification methods are non-invasive and all current permanent identification methods are invasive.

Currently, no non-invasive methods are available that will simultaneously characterise and identify the animals via a single procedure. However, it is possible to combine non-invasive characterisation and identification methods (see Table 1). Such combined procedures must be designated as experiments on animals if they impair the welfare of the animals to or beyond the extent of the lower limit stipulated by law.⁷ These combined procedures can be carried out either before or after the weaning age in accordance with best practices. One example of a best practice is the administration of a local anaesthetic (e.g. lidocaine cream) in the event the ear notching procedure is conducted at a more advanced age.

⁷ Definition according to Article 1(1a) of the Experiments on Animals Act: any use, invasive or non-invasive, of an animal for experimental or other purposes, with known or unknown outcome, or for educational purposes, which may cause the animal a level of pain, suffering, discomfort or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.

In the event that the identification and characterisation procedure is conducted subsequent to the weaning age and the animals are killed without being used for the breeding of a new generation or in an experiment on animals (for example, because they have the wrong genotype), these animals will be registered in the category 'laboratory animals who died or were killed without being used for breeding or in an experiment on animals'.

The various methods are displayed in Table 1.

Explanatory notes to the table⁴

1. Shaven fur

One temporary and non-invasive identification method is the shaving of fur. This method is temporary, as the fur grows back. The method is non-invasive. This method can be used for pups aged two weeks or more that have developed fur, and must be repeated depending on the length of the hairs shaved off and the growth speed of the hairs. To enable shaving of the mouse's fur, the animal must be restrained. Depending on the shaving tool used, the animals may be exposed to ultrasonic noise to a greater or lesser extent.

2. Pen marking

Another temporary and non-invasive identification method is the application of a mark on the skin or fur using a marker or other similar type of pen. The ink used for the mark can be absorbed by the skin or ingested orally via licking, which may interfere with the study. The animal must be restrained in order to apply a recognisable mark and this procedure must be regularly repeated due to fading of the mark, for example, due to the animal licking it.

3. Subcutaneous ink marking

Applying a subcutaneous ink marking is not the same as tattooing, as a tattoo is applied between the epidermis and the dermis. The injection of the ink is painful. Furthermore, the ink can fade. The substances in the ink can cause interference in the study. To enable application of the ink, the animal must be restrained.

4. Tattooing

Application of a tattoo is painful. The ink can fade. The substances in the ink can cause interference in the study. The animal must be restrained in order to apply a tattoo.

5. Metal ear tag

Application of an ear tag is invasive. Among other risks, there is a possibility of infection of the auricle, development of scar tissue and tearing of the ear. If an MRI scan of the animal is required, the metal ear tag must be removed, which requires another procedure to be performed on the animal. Ear tags can only be applied *after* the weaning age and the animal must be restrained in order to apply the ear tag.

6. Plastic ear tag

Application of a plastic ear tag is invasive. In comparison to a metal ear tag, the risk of infection of the ear canal, development of scar tissue or tearing of the ear is lower. Plastic ear tags are available in extremely small sizes, which reduces the likelihood of the mouse getting the tag caught on objects in the cage in comparison to metal ear tags. The animal must be restrained in order to apply the ear tag.

Plastic ear tags do not have to be removed in order to perform an MRI scan of the animal.

7. Subcutaneous microchip

The application of a subcutaneous microchip that is detectable by a reader (as is often used for household pets) is invasive and can result in infection and the development of connecting tissue. The chip can migrate throughout the body, although this risk is reduced in the case of a recently developed extra-small microchip that can be applied at the point at which the tail joins the body (proximal tail tip), for which a substantially thinner needle is required. The animal must be restrained in order to implant the microchip.

8. Droppings

One non-invasive method of obtaining samples for characterisation is the collection of droppings. For this purpose, the animal must be temporarily or permanently housed in an individual cage to ensure that the droppings belong to that specific animal.

9. Oral mucosa, 10. Rectal swab, 11. Hair with hair follicle, 12. Aqueous humour

The sampling of body material in the form of oral mucosa, a rectal swab (using a cotton bud across the anus), the removal of a hair with the follicle attached or the sampling of aqueous humour are classified as non-invasive methods. The animal must be restrained in order to sample the body material.

13. Toe clipping and 14. Ear notching/punching

The animal must be restrained in order to carry out the toe clipping, ear notching or ear punching. As is discussed in this advisory report, no straightforward conclusion can be drawn concerning the impact of toe clipping and ear notching on animal welfare based on existing scientific publications. Further description of this discussion can be found in this advisory report.

15. Tail clipping

Tail clipping involves the removal of a small part of the distal tail. Although this is a reliable method for obtaining DNA for characterisation, this method does not leave an identifying mark. Therefore, an extra procedure must be carried out in order to identify the animal. The animal must be restrained in order to clip the tail.

16. Individual cages

In order to identify specific animals following characterisation, they can be put in individual cages identified with a simple marking once a tissue sample has been taken. As social housing of rodents is standard practice, individual cages cannot be used as a permanent method of identification within the current system.



In-ear identification with microtattoo equipment

Image from Richard and Anne Boutet, Québec, Canada)



Place of insertion (gray circle) of the needle (microtattoo) toe mark

Source: report of Federation of European Laboratory Animal Science Associations Working Group on animal identification)

Table 1: Existing identification and characterisation methods⁸

		Identification		
		Yes		No
		Non-invasive	Invasive	
Characterisation	Yes	Non-invasive (8, 9, 10, 11,12) + (1 or 2)		8. Droppings 9. Oral mucosa 10. Rectal swab 11. Hair with follicle 12. Aqueous humour
	No	1. Shaven fur 2. Pen marking 16. Individual cages (temporary) ⁹	13. Toe clipping 14. Ear notching/ punching	15. Tail clipping

⁸ With the exception of methods 8 and 16, the animals must be restrained in order to carry out the procedure.

⁹ Non-invasive in accordance with the applicable definition, although involves a certain degree of impairment of animal welfare.

The aforementioned FELASA guidelines concerning the refinement of methods to characterise genetically modified animals recommend selecting a method that achieves both objectives without the need for another method. This is only the case for toe clipping and ear notching/punching (13 and 14).

Combining non-invasive characterisation methods with non-invasive identification methods will result in more frequent handling, restraint and marking of each individual animal. Furthermore, it increases the risk of the markings being lost or degraded, which in turn increases the risk of confusion of animals.

Publications show that restraining animals causes stress and can even be classified as a maximum stressor, given that the effect on the heart rate, motility and body temperature caused by restraining the animal is not further exacerbated by the subsequent tail clipping, ear notching or oral/rectal swabbing procedure.¹⁰ This information therefore does not support increasing the frequency of handling and restraining animals for the purposes of repeatedly marking and remarking animals, despite it being plausible that the number of repetitions, the method of restraint and the time between restraint procedures may partly determine the eventual cumulative total of discomfort caused by the repeated restraint and subsequent procedure.

In order to minimise discomfort for the animal, the FELASA working group recommends using the least invasive method that provides a sufficient DNA sample in terms of quality and quantity. In this regard, the NCad would like to emphasise that the quantity required is partly dependent on the DNA isolation and analysis techniques used.

The FELASA working group also recommends taking the samples from animals at as early an age as possible and taking as little tissue as possible. This recommendation is based on the progressive ossification of the skeleton and development of the nervous system as the pups get older.

In the event that identification alone is required

In some cases, the animals only need to receive an identification marking. Both invasive and non-invasive methods can be used to do this. The non-invasive methods are temporary and either require repetition or the animal(s) in question must be kept in individual cages. The methods of permanent identification are all invasive. See the table in the appendix for more information.

¹⁰ - Cinelli P, Rettich A, Seifert B, Burki K and Arras M. Comparative analysis and physiological impact of different tissue biopsy methodologies used for the genotyping of laboratory mice. *Lab Anim* 2007; 41: 174-184.

- Dahlborn K, Bugnon P, Nevalainen T, Raspa M, Verbost P and Spangeberg E. Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. *Lab Anim* 2013; 47: 2-11.

Alternative methods

Technological innovation provides opportunities to develop other methods of identifying laboratory animals.

Identification via recognition of biometric characteristics has been used for quite some time in a variety of areas, such as facial recognition of people for border controls. The effectiveness of facial recognition as an identification technique is based on the fact that biometric characteristics are unique, are personal and undergo little to no change over time. It has been found that computer algorithms can recognise pigs solely by their noses, rendering ear tags or chip implants unnecessary. Zebra fish and mice can also be individually identified in this way, provided they are kept in small groups. As yet, current technology is unable to identify mouse pups, although further development and refinement of 'facial recognition' technology may enable identification of mice or mice pups in future, which would make it a viable alternative for invasive identification methods.

In a 2008 report, Norecopa¹¹ asserted that ear prints could be a possible identification method. Ear prints are based on recognition of the patterns of blood vessels in the ears of mice. This method was being developed at a Swedish location operated by a private entity, and in 2007, researchers at this location published a report for the UK-based NC3Rs.¹² In this report, the researchers concluded that further development was required in order to completely eliminate the risk of incorrect or non-identification. During preparation of this advisory report, we enquired about this method and found that it has not been further developed and has never been used at any scale.

What has the Ministry of Agriculture, Nature and Food Quality (previously the Ministry of Economic Affairs) already investigated?

In 2016, upon request by the Ministry of Economic Affairs, The Netherlands Organisation for Health Research and Development (ZonMw) investigated the degree to which toe clipping is practised and presented a proposal for a separate call to develop an alternative method to toe clipping.¹³

¹¹ Norecopa is the Norwegian national platform set up to make progress on the 3Rs (Replace, Reduce, Refine).

¹² Cameron J, Jacobson C, Nilsson K, Rognvaldsson T. Identifying laboratory rodents using earprints. London: NC3Rs. This article is no longer available via the online database of the NC3Rs.

¹³ ZonMw – Survey of methods employed for the characterisation and/or identification of rodents, June 2016.

Table 2¹³: Survey to determine how many institutes practise distal phalanx biopsy (Source: Professional Group of Animal Welfare Officers, June 2014). The survey was sent to all permit holders (approx. 80). These questions were only relevant to some of the permit holders, because they regularly carry out research and/or breeding activities involving rodents (ZonMw report, 2016).

Respondents	Number of respondents	Phalanx biopsy permitted	Phalanx biopsy practised	Phalanx biopsy not practised
Universities, national institutions (including KNAW), businesses (including animal breeders)	16	13	10	6

Although the Ministry of Economic Affairs only requested information about the scale of the practice of toe clipping, ZonMw also examined the practice of ear notching. The systematic literature study conducted by SYRACLE in 2016 and published in 2017¹⁴ concluded that, based on the existing scientific evidence, an impact on animal welfare stemming from toe clipping or ear notching could neither be confirmed nor precluded. In response to this uncertainty concerning the degree of discomfort to the animals, the Ministry of Economic Affairs sought to restrict the practice of toe clipping to the greatest extent possible in line with the precautionary principle.¹⁵ This principle states that, if no definitive conclusion can be reached concerning the harm caused by a procedure, any possible serious harm must be prevented and that the burden of proof lies with the party that wishes to carry out the procedure.

Via a literature study, ZonMw also conducted further research of a number of alternative characterisation methods, such as methods using oral mucosa, rectal swabs, saliva, hair and stool. All of these genetic characterisation methods require a separate identification method to be applied. No conclusive information is available concerning scientifically substantiated advantages and disadvantages associated with these methods in comparison to other methods and the degree of discomfort caused.

¹⁴ <https://europepmc.org/article/pmc/pmc5700778>

¹⁵ Letter dated 15 December 2016 from the Minister for Agriculture to the Lower House of the States General (32 336, Animal Procedures, No. 64).

The NCad's contribution

Via a range of different bodies at both the national and the international level, the NCad made enquiries to determine which methods can be used for characterisation and identification. Among other parties, the NCad approached a number of major European institutions in the field of genetic modification and animal breeding, the national committees (hereinafter 'NCs') of all EU Member States and a number of animal breeding coordinators in the Netherlands.

Situation at both Dutch and international universities that use a substantial number of genetically modified animals

The NCad approached a number of universities in both the Netherlands and in Europe that use a substantial number of genetically modified animals, and asked them which characterisation and identification methods they use. These enquiries found that both ear notching and toe clipping are practised, although ear notching is practised to a slightly lesser extent.¹³ The respondents often used the same arguments to justify the use of one method over the other. These arguments¹⁶ relate to the degree of discomfort and stress, whether or not the animals can do without the mutilated body part and the age at which the procedure is carried out. Respondents that practise toe clipping state that animals need intact ears for the purposes of communication, while those that practise ear notching state that the animals need their toes to enable them to climb adequately.

Application in other countries

Via the NCs, the NCad carried out an exploratory study of the identification and characterisation methods practised in other EU Member States. The NCad received responses from nine different countries. The results are displayed in Table 3.

¹⁶ Schaefer et al. (2010); Lab Anim 44:7-13, Paluch et al. (2014); J Am Assoc Lab Anim Sci 53:132-40, Dahlborn et al. (2013); Lab Anim 47:2-11

Table 3: Identification and characterisation in EU Member States

Note: No genetically modified animals are bred in Bulgaria. Fewer than 100 genetically modified animals are bred in Croatia every year.

Country	toe clipping status ¹⁷	alternative
Bulgaria	toe clipping not practised	multiple alternatives mentioned: numbering of cages, shaving fur on various parts of the body, a numbered ring on one of the extremities ¹⁸
Germany	permitted because alternatives also result in impairment of animal welfare	
Estonia	toe clipping not practised (prohibited by law)	For characterisation: oral mucosa or hair follicle (not preferred due to unreliable results). For identification: ear tags.
United Kingdom	practised extremely rarely	usually ear notching.
Ireland	toe clipping not practised ¹⁹	ear notching and sometimes tail clipping ²⁰
Croatia	toe clipping not practised	Ear notching if necessary. Tail or fur marking for identification only
Norway	permitted if certain conditions are complied with ²¹	multiple alternatives mentioned: ear notching, tail clipping, tattoos, microchips, marker pens, oral mucosa, hair follicle, stool
Austria	permitted if valid reasons are stated	no preference for a particular method: for each method, the reasons for its selection must be stated and characterisation must be performed using as little material as possible: tail clipping is not preferred
Czech Republic	permitted if other methods are not possible.	Ear clipping, because this is quick and also enables characterisation. Ear tags, as they can be applied quickly, have higher visibility and – if applied correctly – are permanent.

¹⁷ Whether toe clipping is prohibited in the countries in which it is not practised is unknown.

¹⁸ Information provided by a Dutch permit holder that also practises the method shows that the method was not reliable with young or very young animals that were still growing, as the animals were able to remove the ring with relative ease. With neonates, rings cannot be used as an identification method due to the growth of the paw.

¹⁹ The practise is not officially prohibited, although in principle, it also has not been officially approved as the method is considered too cruel.

²⁰ Not an identification method, hence a separate identification method must be performed.

²¹ If it is necessary to collect a large quantity of tissue from an animal aged between 1 and 10 days, then this must be clearly specified in the application. If the DNA material must be taken prior to the weaning age or there are practical or economic reasons to remove animals with an undesirable DNA profile from the colony as quickly as possible.

Ethical aspects

The issue of genotyping and characterisation involves a variety of ethical aspects (in addition to the general ethical aspects concerning the use of laboratory animals, which are not included in the scope of this report).

Toe clipping, ear notching and every other invasive method causes impairment of the animal's physical integrity and welfare. One consideration involved in the ethical assessments relating to animal procedures is that a certain degree of discomfort, harm and physical impairment of the laboratory animals is condoned for the purposes of certain scientific and/or social objectives.

It is the moral responsibility of every individual researcher and permit holder to meticulously assess all considerations relating to identification and characterisation when initiating and designing experiments involving laboratory animals, and not just the ethical assessments required by law.

In addition, the instrumental use of animals as laboratory animals involves a responsibility to do everything possible (to a proportional extent) in order to limit the impairment of physical integrity and animal welfare to the greatest extent possible. This means that the biomedical research sector has a moral duty – possibly reinforced by policy measures – to accelerate the search for innovative non-invasive identification and characterisation methods.

Although it is outside the scope of this advisory report, the NCad wishes to add the following advice:

Morally speaking, this infringement of the integrity and the welfare of the animal must be taken into account during all ethical assessments relating to animal studies, including the effect of other procedures on both of these aspects. In other words, consideration must be given to whether extra procedures and/or interventions are necessary for the effective conduct of research projects using laboratory animals, as well as assessment and comparison of the various available methods.

However, decisions relating to identification and characterisation are not currently included in the ethical assessment required by law in order to determine whether or not to issue a project permit. This is partly due to the fact that, in the Experiments on Animals Act, toe clipping and ear notching for the purposes of identification – which also provides the tissue required for genetic characterisation – are defined as part of the customary housing and care procedure rather than as an experiment on animals in itself.

The NCad believes that this aspect should also be taken into consideration during evaluation of the Experiments on Animals Act.

Conclusions

As previously observed by ZonMw and as demonstrated by our findings, a great deal of contradictory information has been published concerning the impact on animals of the various characterisation and identification methods. The conclusion of a systematic review²² published in 2017 is that little to no well-founded scientific evidence exists that reliably estimates the degree of impairment of animal welfare resulting from the practices of toe clipping and ear notching. However, this does not mean that the practices do not impair animal welfare. Evidence that these methods cause impairment of the animal's welfare does exist, although none exists to show whether one technique causes greater impairment than the other.

The Ministry of Agriculture, Nature and Food Quality would only be willing to ban the practise of toe clipping provided an effective alternative is available. However, based on the aforementioned information, there are insufficient scientific grounds to either ban the practice of toe clipping and/or ear notching or permit both. Other characterisation or identification methods also involve discomfort for the animal. For this reason, the NCad has decided that no Code of Practice will be derived from this advisory report.

During the preparation of this advisory report, the NCad found no additional scientific evidence to suggest that the conclusions of the 2017 systematic review should be re-evaluated. However, the NCad recognises and gives due consideration to the intrinsic value of animals when formulating its advice, and for this and other reasons observes the precautionary principle.

The NCad has established that no other non-invasive methods currently exist that both enable a sample to be collected for the purpose of genetic characterisation and apply a unique and durable identification mark to the animal via the same procedure.

Non-invasive methods do exist for the purposes of both genetic characterisation and identification, and combination of these methods is possible. However, this means two procedures must be conducted rather than one and the identification method will have to be regularly repeated. The NCad considers it plausible that a combination of non-invasive methods will impair the animal's welfare to a lesser extent than the invasive methods. It must be noted that the restraint of animals also causes a certain degree of stress and that the animal will have to be restrained every time an identification mark is applied or reapplied. However, methods to reduce this stress do exist, such as animal training. The responsibilities of the parties involved prompt meticulous evaluation of the most suitable identification and characterisation methods for each individual project, with

²² Wever, K. E., et al. (2017). 'A systematic review of discomfort due to toe or ear clipping in laboratory rodents.' *Lab Anim* 51(6): 583-600.

careful consideration given to aspects such as infringement of the animal's physical integrity and welfare and the quality of the science produced.

Given the large number of animals that are both identified and characterised every year, the NCad believes that attention should be paid to the development of combinations of sustainable and non-invasive identification and characterisation techniques that involve little to no infringement of the animal's physical integrity and welfare.

The NCad believes that further research into innovative identification and characterisation methods for laboratory animals is advisable, such as individual facial recognition and DNA isolation/analysis techniques.

The NCad's advice to the Ministry of Agriculture, Nature and Food Quality and the Ministry of Education, Culture and Science is to set up a concrete project with the objective of implementing the aforementioned recommendations. Furthermore, during evaluation of the Experiments on Animals Act, attention should be devoted to the impact on the physical integrity and welfare of laboratory animals of procedures that are not included in – but are directly related to – the official definition of 'experiment on animals'.

Appendix: Summary of identification and characterisation methods

	1	2
Method	Shaven fur	Pen marking
Explanation	Temporary and non-invasive identification method. This method is temporary, as the fur grows back. The method is non-invasive. Can be used for pups aged two weeks or more that have developed fur, and must be repeated depending on the length of the hairs shaved off and the growth speed of the hairs. The animal must be restrained. Depending on the shaving tool used, the animals may be exposed to ultrasonic noise to a greater or lesser extent.	The application of a recognisable marking on the skin or fur using a marker or another similar type of pen is a temporary and non-invasive identification method. The ink used for the mark can be absorbed by the skin or ingested orally via licking, which may interfere with the study. The animal must be restrained in order to apply the marking and this procedure must be regularly repeated due to fading of the mark, for example, due to the animal licking it.
Identification	X	X
Characterisation		
Temporary	X	X
Permanent		
Before weaning age	X	X
After weaning age	X	X
Invasive		
Non-invasive	X	X

8	Droppings	One non-invasive method of obtaining samples for characterisation is the collection of droppings. For this purpose, the animal must be temporarily housed in an individual cage to ensure that the droppings belong to that specific animal.	7	Subcutaneous microchip	The application of a subcutaneous microchip that is detectable by a reader (as is often used for household pets) is invasive and can result in infection and the development of connecting tissue. The chip can migrate throughout the body, although this risk is reduced in the case of a recently developed extra-small microchip that can be applied at the point at which the tail joins the body (proximal tail tip), for which a substantially thinner needle is required. The animal must be restrained in order to implant the microchip.	6	Plastic ear tag	This method is invasive. In comparison to a metal ear tag, the risk of infection of the ear canal, development of scar tissue or tearing of the ear is lower. Plastic ear tags are available in extremely small sizes, which reduces the likelihood of the mouse getting the tag caught on objects in the cage in comparison to metal ear tags. The animal must be restrained in order to apply the ear tag. Plastic ear tags do not have to be removed in order to perform an MRI scan of the animal.	6	Method	Explanation
										Identification	
										Characterisation	
										Temporary	
										Permanent	
										Before weaning age	
										After weaning age	
										Invasive	
										Non-invasive	

	15	14	13	9, 10, 11, 12	Method
	Staarstkniip	Oorkniip	Teenknip	Wangslimvlies (9), rectale swab (10), Haar met haarzakje (11), oogvocht (12)	Explanation
	Tail clipping involves the removal of a small part of the distal tail. Although this is a reliable method for obtaining DNA for characterisation, this method does not leave an identifying mark. Therefore, an extra procedure must be carried out in order to identify the animal. The animal must be restrained in order to clip the tail.	See toe clipping.	The animal must be restrained in order to clip the toe. As is discussed in this advisory report, no straightforward conclusion can be drawn concerning the impact of toe clipping on animal welfare based on existing scientific publications.	The sampling of body material in the form of oral mucosa, a rectal swab (using a cotton bud across the anus), the removal of a hair with the follicle attached or the sampling of aqueous humour are classified as non-invasive methods. The animal must be restrained in order to sample the body material.	
		X	X		Identification
	X	X	X	X	Characterisation
				X	Temporary
	X	X	X		Permanent
	X	X	X	X	Before weaning age
				X	After weaning age
	X	X	X		Invasive
				X	Non-invasive

This is a publication of:

The Netherlands National Committee for the Protection
of Animals Used for Scientific Purposes
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july 2020 | 20103149