Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification

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Working Party Report

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Abstract

The primary aim of this report is to assist scientists in selecting more reliable/suitable identification (ID) methods for their studies. This is especially true for genetically altered (GA) animals where individual identification is strictly necessary to link samples, research design and genotype. The aim of this Federation of European Laboratory Animal Science Associations working group was to provide an update of the methods used to identify rodents in different situations and to assess their implications for animal welfare. ID procedures are an indispensable prerequisite for conducting good science but the degree of invasiveness differs between the different methods; therefore, one needs to make a good ethical evaluation of the method chosen. Based on the scientific literature the advantages and disadvantages of various methods have been presented comprehensively and this report is intended as a practical guide for researchers. New upcoming methods have been included next to the traditional techniques. Ideally, an ID method should provide reliable identification, be technically easy to apply and not inflict adverse effects on animals while taking into account the type of research. There is no gold standard method because each situation is unique; however, more studies are needed to better evaluate ID systems and the desirable introduction of new and modern approaches will need to be assessed by detailed scientific evaluation.

Keywords: Animal welfare, biopsy, refinement, rodent identification, toe clipping

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Rodent identification and animal welfare

The aim of this Federation of European Laboratory Animal Science Associations (FELASA) Working Group was to identify best practices for rodent identification in different situations and assess their implications for animal welfare. In general, the new EU Directive does not apply to practices undertaken for the primary purpose of identification of an animal (2010/63/EU). This means that it is left to the national authorities to decide what methods are acceptable or not. The overall aim of this report is to assist scientists in choosing the best identification method for their studies, and to enlighten legislators in the decision-making process.

Early individual identification is a prerequisite for valid and reliable research using animals; this is especially true in the case of genetically altered (GA) animals. Identification is necessary to link samples, research data and genotype to the individual animal. Loss of identification can render the animal unusable for further breeding and research. Data can also become unusable, which can result in compensatory use of additional animals. Reliable identification is therefore a prerequisite for good science and has important Reduction aspects. Although, identification methods are considered routine procedures, there is often a lack of scientific evidence related to their impacts on animal welfare and research outcome. Therefore, this report is based on best practice and, when available, on the scientific literature.

A wide variety of identification (ID) methods are used, each with different implications on animal welfare. Concomitantly, new and improved methods are being developed. The method of choice generally depends on tradition, study-based reasons and costs. A survey was performed in 2007 on the web-based forum Comparative

Medicine list (COMPMED), and it showed that the most commonly used methods are ear notching/clipping and ear tagging, both in the USA/Canada (ear notch/clip; 10 out of 23 answers, ear tag 11 out of 23) and in Europe (ear notch/clip; 10 out of 19 answers, ear tag 4 out of 19).[1] The least widely used methods were toe clipping and ear tattooing.

All identification methods are brief procedures, involving restraining the animals and result in some degree of discomfort and/or pain. Since these procedures are carried out on a vast number of animals, even minor improvements for the individual animal can lead to a considerable overall Refinement effect. A more expanded version, with practical details on how to perform different methods, will be published on the FELASA homepage.

Animal welfare is generally assessed with a combination of physiological and behavioural parameters. There are reliable parameters to be used for assessment of both acute and chronic effects on animal welfare. However, the identification procedure is often the first restraint to which animals will have been subjected, e.g. at weaning or even before, and therefore the assessment of acute effects of the identification procedure can be difficult to distinguish from the effects of the handling and/or restraint itself.[2,3] Reported long-term negative consequences of identification are increased mortality, systemic diseases, tissue irritation or damage, inflammation and tumours.[4–7] Therefore, a valid assessment of a specific identification method should include both immediate and chronic effects associated with the procedure. In addition, the ease with which the procedure can be performed, as well as the readability and sustainability of the marking over time, should be taken into account when evaluating the different methods. If genotyping is necessary, it can be considered as a true Refinement if the chosen identification method also generates a tissue sample, thus avoiding repeated (invasive) procedures on the same animal.

Acute effects are not only an animal welfare concern, but also a research quality concern. Invasive identification methods necessitate a recovery period before the animals can be used in the study. For example, the expected outcomes of tattooing are oedema and bleeding through puncture holes made by the tattooing instrument. This will temporarily affect the animals' physiology and behaviour which in turn may affect specific experimental parameters.

Analgesia and anaesthesia can be useful because they shorten the recovery period after invasive and painful procedures, e.g. surgery. The small size of the body parts used for identification makes it very difficult to apply local analgesics/anaesthetics. Inhalation anaesthesia must be considered in order to alleviate acute pain during the identification procedure. To eliminate postoperative pain, inhalation anaesthesia must be combined with analgesia. More studies are required to assess if the methodology could be refined to such a level that it would really alleviate the stress to which the animals are subjected during and after the identification procedure. In addition, the success of any method depends on the training and manual skills of the person performing the procedure and care of the instruments used.

In this report, the identification methods are grouped according to whether or not they are

(1) Permanent
(2) Invasive
(3) Generating tissue sample for genotyping.

Rodents are the largest group of mammalian species used in research and testing in the European Union (95%).[8] Therefore, the methods described here focus on rats and mice. However, most methods are also applicable to guineapigs, hamsters, gerbils and chinchillas.

Non-invasive temporary identification methods

Shaving or cutting the fur

Cutting the fur is one of the simplest methods for identifying laboratory rodents. An area on the body, mainly on the back (for visibility without handling), is cut or shaved. The most evident advantages are the ease of application and reading, along with the low cost. On the other hand, this type of ID can only be used to distinguish a limited number of animals.

The only necessary equipment is a fur shaving machine or a pair of scissors. The ease of reading the marking depends on the growth of the fur and the length of hairs that are removed. This method can be used after approximately two weeks of age, when pups have acquired a full coat of fur. The stress associated with this identification method mainly stems from the restraint of the animal. If properly performed, the procedure should not cause any pain to the animal.

Felt tip marker or alcohol-based pens for skin marking and coat dying

If one uses a felt-tip pen or similar markers, it is possible to identify mice or rats by writing marks or numbers on hairless parts of the body, e.g. ears and tail, but also on the skin (back skin, belly, limbs, armpits), especially in neonates or hairless mutants. The numbering or coding options can be increased by using different colours. The method is applicable to all ages, including neonates.

Commonly used dyes for colouring the fur are human hair colours or marking sprays meant for farm animals, but also a felt-tip pen can be used. Fur colouring is not suitable for a large number of animals. The marks are readable from a distance and without handling the animal. However, if neonates are marked on the armpits, handling is necessary in order to identify the animal. Coat dying is applicable as soon as the animals have fur, i.e. after approximately two weeks of age. Both skin marking and coat dying as such are painless but require restraint for application and renewing which can cause a temporary stress in the animal. This increased handling can also have positive effects.[9] These are low-cost but time-consuming methods, permitting identification of a limited number of animals only.

One major problem when using dyes or discolouring substances is their potential toxicity or untoward chemical

burden. These substances can enter the body by ingestion (e.g. grooming) or by diffusion through the skin which can lead to interference with research results.

Invasive temporary identification methods

Subcutaneous injection of ink

The subcutaneous injection of ink differs from tattooing because the ink is injected under the skin instead of into the skin layers. Like all subcutaneous depots of substances, ink also fades after some time (a few hours to a few days). The method provides limited numbering possibilities, although this can be increased by using different colours.

This procedure has two painful components; the insertion of the needle through the skin and thereafter the irritation due to the substance and/or the expansion of a body part as a result of the volume injected. In order to reduce the pain one should avoid using toxic or irritant substances. The footpad or the tail is the most common sites of injection. Leclerq and Rozenfeld reported a local swelling after footpad injection which may have been associated with pain.[10] This procedure requires restraining the animal and training the personnel. Handling is necessary to read markings on the tail and restraint is necessary to read the markings on the footpads.

This method can be used in animals of all ages, including newborns. It does however have a limited use since it only lasts for a short time (days at best). The Working Group does not recommend using this method since it is invasive and painful and only temporary.

Ear tag

Ear tags are available in metal or plastic; these are applied to the ear with special pliers. Tags are available in different sizes, to be used on different species, are pre-numbered and thus allow identification of a very large number of animals.

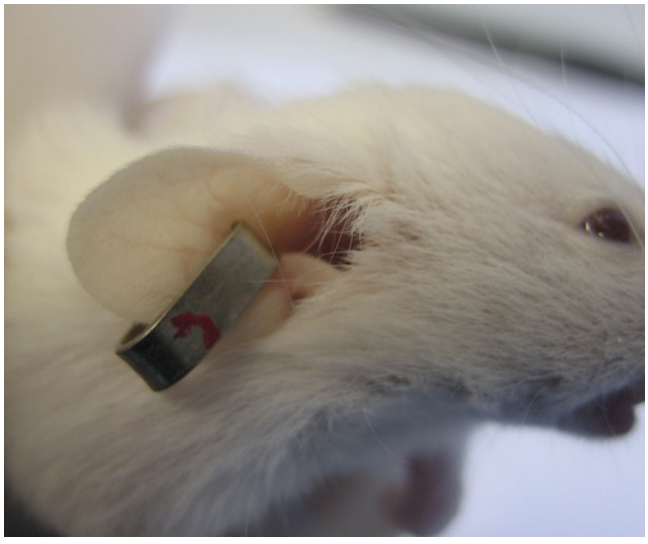

Figure 1 Tag placed correctly on the lower edge of the pinna

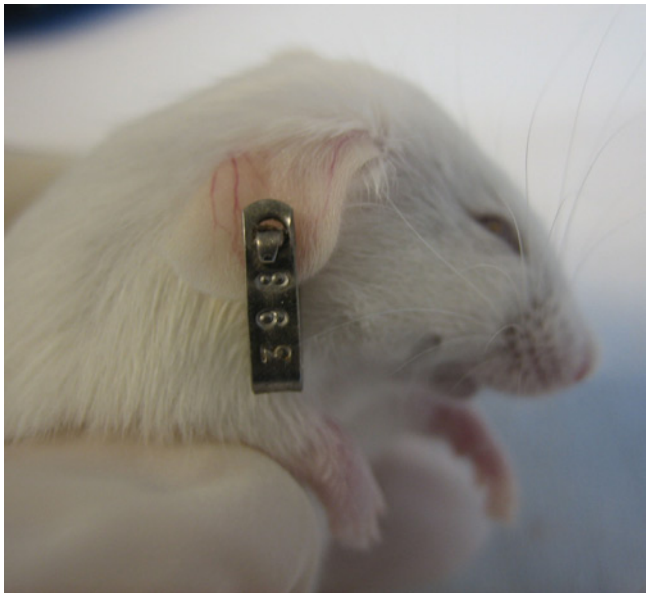

Figure 2 Tag placed in the upper edge of the pinna (incorrect)

The pliers are loaded with a tag and placed in the visible ear (*pinna*). Tags should be placed on the lower edge of the pinna so that they do not bend it (Figure 1). Ears should be checked regularly and in cases of tissue damage or inflammation, the tags need to be removed. Restraint is necessary for proper attachment of the tag and for its subsequent reading when identifying the animal.

This ID method is inexpensive and quick and easy to perform. There is some risk that the animal will lose the tag. The pinna is not developed before two weeks of age and to ensure that the ear is big enough to hold the tag, one need to wait until weaning. Furthermore, in experiments using magnetic resonance imaging (MRI) systems, the metal tags will need to be removed because they will interfere with the magnetic field. The method is painful and requires proper restraining of the animals and therefore they will temporary suffer for a brief period from a combination of pain and discomfort.

How the tag is placed in the pinna is important, with placement in the lower edge being recommended (Figure 1).[11] If the tag is placed on the dorsal side of the pinna, the ear might fold (Figure 2) and this could cause irritation and suffering to the animal.

Metal ear tags (commonly made from a nickel–copper alloy) have been associated with inflammatory and proliferative reactions and neoplasia in mice and rats following months of carrying the tags.[6,7] Similarly, a remarkably high incidence (8.8%) of squamous cell carcinomas has been reported.[4] The appearance of auricular chondritis, in C57BL/6 mice, was claimed to be an autoimmune response to reactive compounds released from the metal tag.[12] In that study, tagged ears contained higher levels of copper and cytokines, compared with non-tagged ears. When different tags were compared in guineapigs, the loss of tags decreased from 45% for metal tags down to 10% with nylon tags.[13] Hence, the risk of irritation and inflammation

seems to be related to the metal in the tags and to the duration for which the animals have to carry them.

Invasive permanent identification methods that do not yield a tissue sample for DNA analyses

Tattoo methods

Several body parts can be used for tattoo identification in rodents, e.g. ears, tail, footpads or toes. The procedure includes penetration of the skin with specific instruments in order to load tattoo ink or paste intradermally. The sensitivity of the body part where the marking is being applied will vary, and this has consequences on the extent of the pain sensation experienced by the animal in connection to the procedure.[14]

The ink must be loaded in the dermis, under the epidermis (the upper layer of the skin) to create a permanent marking. Hence, the skin barrier is disrupted and chemical compounds in the ink can spread via the circulation to the entire body. This poses two types of hazards: those associated with toxicity and those that may interfere with the study. Tattooing needles should always be clean (aseptic), kept sharp and replaced on a regular basis. Tattoo pigments are usually minerals, organic (industrial) or plastic-based pigments. Substances that are toxic or which may interfere with research results should not be used. In MRI studies, there is a specific problem with some tattoo inks.

Tattooing is a permanent method, but the ink may fade and become illegible with time. The number of possible unique identification marks possible varies depending on which method is used, but it can generally be increased by using different colours or by combining different locations. In general, tattooing procedures require training before they can be performed properly.

Ear tattoo

This method requires fully developed ears and a good restraint that fixes the head of the animal is necessary to avoid lateral movement and unnecessary tissue damage during the application. Restraint may be needed to read the tattoo.

One of the two methods for tattooing rodent ears is to use tailor made instruments, typically pliers with revolving heads. The second method available for tattooing rodent ears is the microtattoo system. This involves a pair of forceps with a disposable hypodermic needle on one side and a container with ink paste on the other, and the procedure is monitored through a magnifying glass (Figure 3). The identification consists of combinations of dots which allow for creation of a large numbering system. Generally, only handling is needed to read the ear tattoo.

Ears are considered to be very sensitive organs,[14] and hence tattooing must be considered to be at least moderately painful. Adult rats, instrumented with telemetric cardiovascular transmitters, were marked with three different methods; ear tattoo, ear notching and micro (toe) tattoo, using a crossover design. During the 1–4 h period and the following dark period, the mean arterial pressure was highest in the ear notching group indicating that the pain evoked was still present during 1–16 h after the marking procedure.[15]

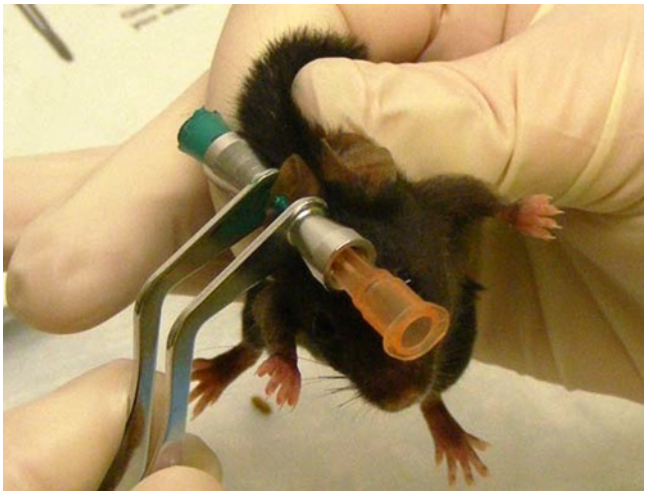

Figure 3 Identification in ear with microtattoo equipment (photograph by Richard and Anne Boutet, Québec, Canada; reproduced with permission)

Tail tattoo

Tattooing on the tail can be performed in two different ways: the microtattoo system or the electric tattoo equipment (similar to that used in humans). Tail tattooing with the microtattoo system should only be applied in young animals before the ossification of the tail (tail ossification occurs between 2 and 3 weeks of age)[16] since it is inserted completely through the tail. Tail tattooing with an electric machine can be performed on adult mice, and rats of all ages if one wishes to write digits or letters. It can also be used in young mice to imprint dots or stripes on the tail. First the ink is applied on the skin and the tattoo needles transfer the ink into the skin layers and the remaining ink is then removed. This method allows for an infinite amount of numbers but needs some prior training in order to apply readable digits/letters. An alternative to the electric tattoo machine is the use of a lancet. It is done manually and produces only coloured dots, which results in a limited amount of numbers. As for the other tattoo methods, good restraint is necessary when tattooing the tail. In general, only handling is necessary to read the tattoo.

The pain, the necessity to restrain and the long duration of the tattooing procedure with the electric tattoo machine (noise, vibrations) cause discomfort to the animals. The manual method using the lancet also causes pain, the extent of which will obviously depend on the skill of the person performing the procedure.

Guillod and Johnson (1990)[17] found that ink tail tattoos caused mild fibrosis in tattooed areas and uptake of ink in regional lymph nodes, but no effects of tail tattooing on food consumption in a long-term study in adult rats. As a result of licking the tattoo, Sørensen *et al.*[18] found ink

Figure 4 Site of insertion (grey circle) of the needle (microtattoo) for toe marking

colour in the faeces of 20-day-old mouse pups after tail tattooing.

Toe tattoo or footpad tattoo

The microtattoo system can also be used to mark a toe or footpad by inserting the needle through the skin of these extremities. The needle should not be inserted through the whole toe or foot, only the pads are marked.

An important advantage of this method is the possibility to identify animals of all ages. Even when the toes of newborns are not yet separated, it is possible to perform toe tattooing. The microtattoo is inserted through the toe pad (Figure 4) or through the footpad.

A lancet can also be used to mark the pads of the toes or of the foot, employing the same method for tail tattoos and a lancet.

All toe or footpad tattoo methods described are considered to be painful, but the use of the microtattoo can be considered as acceptable because the needle used can be adapted to the size of the animal.

Microchip transponder

Electronic radio frequency ID transponders, commonly called microchips, are an effective way to identify laboratory animals. A microchip is inserted subcutaneously, in order for the animal to be identified with a transponder reader. The transponder responds to a low-energy radio signal emitted by a compatible reader, which displays the information (number) from the transponder. The microchip is implanted in the neck or further back, via a special syringe. The microchip system is a permanent ID method and allows for identification of an infinite number of animals.

Most readers can be connected to a computer which make it possible to collect a variety of data from a specific animal and transfer these directly to databases. Although the application is initially time consuming, the microchip has the advantage over other methods in that identification errors (except for incidental chip loss) are excluded. While it is

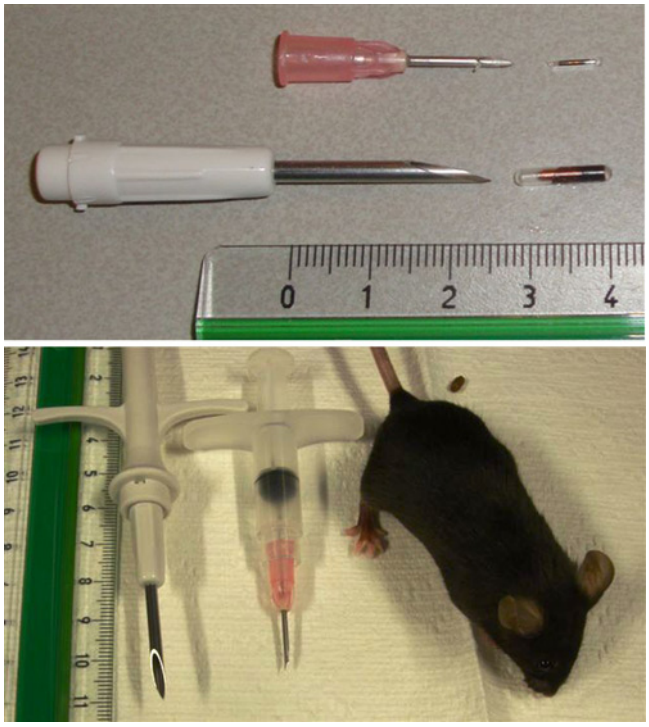

Figure 5 Size of microchips suited to mice (1 × 6 mm) and rats (2 × 12 mm) (top picture) and the size of the needles (12G, to the left and 18G, to the right) in relation to a fully grown C57BL mouse (bottom picture)

true that microchips can disappear, relocate or break, this risk appears to be relatively low. Rao and Edmondson (1990)[19] monitored 140 implanted mice for two years and found that 2% of the mice lost their chips, and 2.8% of the chips failed to transmit data.

The suitable time for microchip insertion depends on the size and body weight of the animal rather than age. The larger chip (12 × 2 mm) (Figure 5, top picture) is most suitable for animals ≥50 g and should not be used before adulthood in mice – if at all. The smaller chips (6 × 1 mm) are more suitable for mice (Figure 5, top picture). The manufacturer of the smallest microchip (6 × 1 mm) claims that it can be used in mice from five days of age, but according to Castelhano-Carlos *et al.*,[20] this is not recommended. Inhalation-anaesthesia is appropriate for the microchip implantation in all rodents.[21] It is recommended to close the wound, after insertion.

For proper application and correct positioning of the chip, some training is recommended. In general, handling, but not restraint, of the animal is sufficient to read the ID. For the small microchips, the reading distance is very short and thus it is necessary to touch the animal with the reader to collect the signal.

A recent study on five-day-old mouse pups showed that microchip (size 6 × 1 mm) injection resulted in a stronger reaction (sudden movements, urination and vocalization), compared with distal phalanx removal or toe tattooing performed at the same age.[20] None of the methods exhibited any postnatal effects. The authors recommended the use of toe clipping in young pups and microchips only after weaning.

In the long-term microchips can cause inflammation and fibrous tissue growth. Since they are implanted via injection, this is a procedure known to increase tumour risk. A causal link between microchips and cancer has been postulated to exist in rats and mice. Five out of eight articles reported that 0.8–4.1% of laboratory mice and rats developed malignant tumours around or adjacent to the implanted microchips.[22] In one of these eight studies, the investigators used a genetically modified line (p53+/− mice) that was prone to develop cancer, and 10.2% of the mice developed tumours[5] and in several cases these tumours also metastasized. The tumours generally occurred in the second year of the studies, in middle aged or older animals. However, in the Blanchard study, the heterozygous p53+/− mice developed fast-growing cancers before six months of age.[5] Taken together, microchip implantation appears to increase the risk of developing tumours. This is important to bear in mind when undertaking long-duration cancer research studies in rats or mice. One needs to consider also whether a foreign body like a microchip can affect immunological and skin studies (because of inflammation) or disturb image analysing techniques.

This is likely to be the most expensive identification method (costs for reader and transponders), and anaesthesia equipment may be necessary as well. Finally, the different types of transponders often transmit at different wavelengths and thus require different readers. This may need to be considered before transporting chipped animals between laboratories.

Invasive permanent methods of identification yielding tissue for DNA analyses

Ear notching

Ear notching as an identification method is generally considered to be easy to carry out and read and the necessary trauma to the animal is minimal in a properly executed procedure. The procedure can be applied on mice and rats. Punching or 'notching' holes in the ears requires a specific instrument. The choice of puncher is extremely important; there are punchers (mostly cheap ones), which pinch rather than cut and they should not be used.

The location of the holes must be accurate and done according to a chart/system in order to ensure a valid identification. The withdrawn tissue remnant(s) can be used for genotyping. The punchers must be completely cleaned between animals to avoid any DNA cross-contamination. The marking can be read from a distance but it may be necessary to pick up the animal from the cage. Metal ear punchers allow identification of the laboratory rodents by notching one or several holes in the pinna of the ear, at the edges of the pinna and/or in the middle. The numbering system of ear notching/punching allows identification of maximally a few hundred animals.[23]

In general, this can be considered a permanent method but there have been reports that the pinna can heal after several months depending on the size of the hole. Ear notching is painful and requires proper restraint of the animals and therefore they will briefly suffer from a combination of pain and discomfort. In order to read the ID it is necessary to pick up the animal, but generally restraint will not be needed. Training is required to learn the proper notching technique.

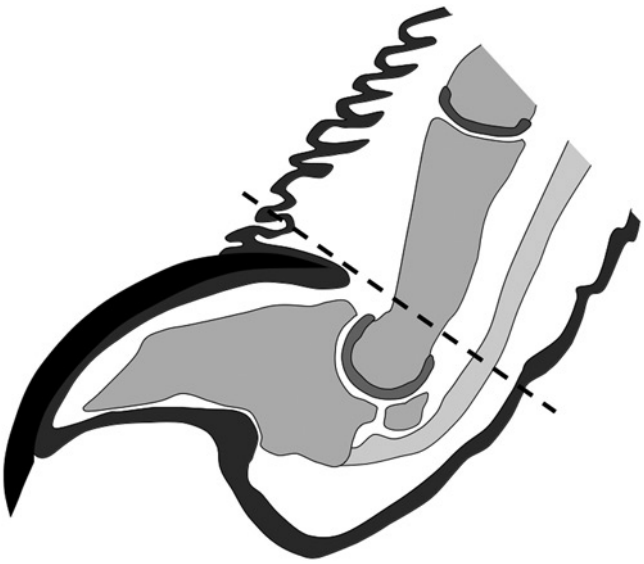

Figure 6 Schematic picture of the site of distal phalanx removal

Cinelli *et al*. (2007)[3] showed that different methods for biopsy collection in mice (tail biopsy, ear punch, mouth swab, rectum swab, hair collection) had the same effects on telemetrically recorded heart rate, motor activity and core body temperature as restraint alone. These parameters returned to normal levels one hour after the biopsy collection. In rats, during the first hour after the marking procedure, ear notching resulted in a higher blood pressure and heart rate responses than toe tattoo, which may simply be due to the fact that less restraint was needed for the latter procedure.[15] Using ultrasonic vocalization as a measure of pain, there was no difference in the response to ear notching and tail snip in mice.[24]

Distal phalanx removal

In distal phalanx removal, the entire distal phalanx of a toe is removed with sharp scissors from mouse pups around seven days of age. The cut is placed at the very distal part of the second phalanx (Figure 6) to remove the entire nail bed. In adult animals the identification is detected as a missing nail or tip of a toe on a paw. The method has been used in rats but is not recommended due to its long-term effects, e.g. impaired grip strength at weaning.[25] Therefore, the method will only be described for mice in this report. A similar, non-refined method generally known as 'toe clipping', where a larger part of the toe is removed, is sometimes used (because it makes the identification easier). However, the use of this non-refined method is strongly discouraged by the Working Group. National legislation within some European countries may actually prohibit the use of this method.

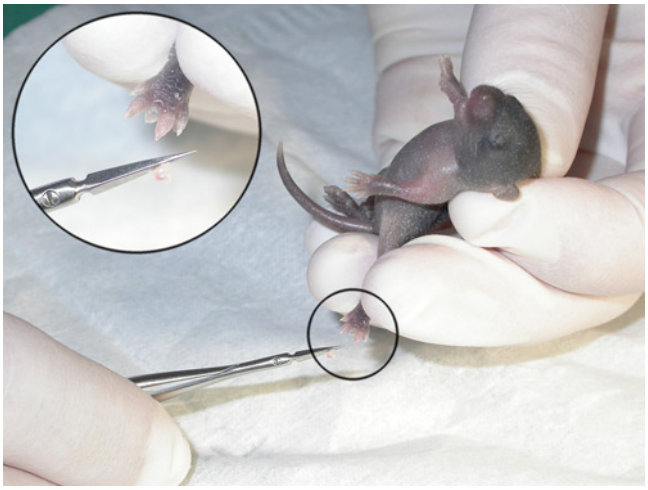

Figure 7 Restraining and cutting of the distal phalanx of a seven-day-old mouse pup (photograph by Dagmar Schaefer, Zürich, reproduced with permission)

The young pups are picked up and held by the nape of the neck in very much the same manner as the mother carries the pups. In addition, it is necessary to restrain the leg (Figure 7) to avoid sudden movements that might result in an incorrect cut. The scissors are opened and placed against the toe from below, in order to make it easier to see and cut the correct amount, which is 2–3 mm in a seven-day old pup (D Schaefer, personal communication). The removed phalanx biopsy can be used for DNA analysis (enough tissue for polymerase chain reaction).

The scissors used for this procedure should be small and sharp, e.g. ocular microsurgical scissors (Figure 7). The Working Group recommends a maximum of one toe per paw, i.e. four toes per animal, to be cut. However, since pups are marked while still in their native litter, males and females within the same litter can be given the same identification since they can be distinguished by sex. This can reduce the number of digits that need to be removed. The reading of the marking (a missing nail or tip of a toe on a paw) requires that the animal is picked up from the cage and held on a surface, such as the arm, to examine the paws. Sometimes restraint may be necessary.

This is a permanent method, if performed correctly, and it will not become illegible over time, which is a risk with some other permanent methods (e.g. tattooing and ear notching). The distal phalanx removal should only be performed on very young animals, Schaefer *et al.* (2010)[26] found that phalanx removal at day 7 was preferred over day 3 because at the younger age it was difficult to cut the correct amount since the toes were so small. Castelhano-Carlos *et al.*[20] successfully performed distal phalanx removal at five days of age and Spangenberg *et al.*[11] at six days of age. Already at 12 days of age the pups are very active and it becomes difficult to perform the phalanx removal with precision. An incomplete removal of the distal phalanx can lead to the regrowth of the digit.[27–30] It seems to be important to remove the entire nail bed to avoid regrowth. By around day 18, the phalanges have become ossified[31] which would make the procedure significantly more painful. Hence, the current knowledge shows that the age of 5–7 days (counting the day of birth as day 0) is a preferable time frame for removing phalanges for identification and genotyping of mice. There is no scientific support for successfully, and without inflicting more pain, performing the phalanx removal at later ages.

If the phalanx is removed correctly, the young pups display little or no reaction; mainly paw withdrawal, during or after the procedure.[20,26] The restraint mimics the mother's handling of the pups and might therefore be less stressful than the common techniques of restraining adult animals. There is usually a drop of blood on the cut tip but no further bleeding.[26] If further bleeding should occur, it can be stopped with a styptic or a haemostatic pencil. Schaefer *et al.*[26] found no effect of phalanx removal on grip strength nor did it cause hyperalgesia (tested at 12 weeks of age) when phalanges had been removed on day 7, but grip strength was impaired when the phalanges were removed on day 3. It is likely that too much of the toe was cut on day 3 due to the small size.[26] No effects on climbing abilities at weaning,[11] or as adults,[20] were found in mice with distal phalanx removal.

Today this method is in fact the only permanent identification of a pup with a simultaneous tissue sampling for genotyping. Because of the early genotyping, a rapid and early selection of animals needed for studies or further breeding is possible which eliminates the costs of housing redundant animals. The recommendation of the Working Group is to use this method only in combination with genotyping and exclusively for young pups. Other methods are options only for marking to only mark young animals when no biopsy is needed.

New methods

The first method described below has recently become commercially available (2010) and is a modification of the classical ear tag. The second method is also commercial available. The final two methods described use completely new approaches to identifying rodents. However, they are not yet commercially available.

Mini-ID Ear tags (http://www.zonotid.com/)

A recent alternative to traditional ear tags is a lightweight plastic tag (0.07 g) that has a 2D barcode etched onto a titanium plate attached to the plastic tag. It is read using a barcode reader similar to the microchips. Its application to the pinna is similar to that done with the traditional tags. This tag type is lighter and should therefore reduce the risks of infections or inflammation in the ear caused by irritation from a heavy tag. It does not have the loop shape of a metal ear tag which minimizes the risk of it becoming entangled. Further, the plastic material reduces the risk of an allergic reaction to the tag. Like other ear tags, this

Table 1 Overview of available identification methods for rodents; issues related to both techniques and the animals

	Concerning the technique							Concerning the animal	
Identification method	Permanent/ Temporary	Specific skills/ training	Number of codes	Age for application (from)	Anaesthesia*	Aseptic measures†	Sample for DNA	Pain/ Discomfort at application	Handling/ restraint at reading
Shaving/cutting the fur	T	No	5	2 weeks	No	No	No	D	None
Skin marking	T	No	10/colour	All	No	No	No	D	None/H
Coat dying	T	No	5/colour	2 weeks	No	No	No	D	None
Subcutaneous ink injections	T	Yes	~8/colour	All	No	Yes	No	P	H/R
Ear tag	T	Yes	Infinite	Weaning	No	Preferable	No	P	R
Ear tattoo	P	Yes	Hundreds	Weaning	No	Yes	No	P	H/R
Tail tattoo	P	Yes	Hundreds	Weaning	No	Yes	No	P	H
Tail microtattoo	P	Yes	Infinite	~2 weeks	No	Yes	No	P	H
Toe/foot pad tattoo	P	Yes	Hundreds	All	No	Yes	No	P	R
Microchip transponder	P	Yes	Infinite	Depends on body size	Yes	Yes	No	P	H/R
Ear notching/ punching	P/T	Yes	Hundreds	2 weeks	No	Preferable	Yes	P	H
Distal phalange removal	P	Yes	Hundreds	4–8 days	No	Preferable	Yes	P	H

For detailed information see corresponding section in the report
The first three methods mentioned in the table are non-invasive
*Analgesia explained in welfare paragraph
†Depending on body part, see tattoo paragraph

method can be used from weaning and allows identification of an infinite number of animals. Other types of ear tags are also becoming available on the market.

Microtransponder p-Chip

This is a new method with a radiofrequency identification tagging that uses 500-μm, light-activated microtransponders implanted subcutaneously into the ear or tail of mice. According to Gruda *et al.* (2010)[32] the preferred location for implanting is in the side of the tail, because implantation at this site was reported to be simple to perform and was associated with shorter implantation times and a higher success rate compared with the ear. They claim that the main benefits of using light-activated microtransponders over other identification methods are their small size which minimizes stress to the animals during implantation.

A biometric approach to laboratory rodent identification

This new technique uses the blood vessel pattern of the pinna of an animal as biometric identification. Each animal has an individual blood vessel pattern, like a fingerprint. Images of the ears of all animals that are to be identified are taken. Later when an animal has to be identified, a new picture is taken of the ear and this is compared with the information stored in the database to identify that individual.[33] It can likely be used from weaning, when the ears are large enough. Since the blood vessel patterns are unique for each individual, this technique should allow identification of an infinite number of animals.

Luminescent Micro Tattooing LMT

With this method, very small luminescent pigments are applied to the skin of the animal, such as on the tail base or the ear, in the form of dot writing (like Braille). It is called 'Luminescent Micro Tattooing LMT'. It applies the code by means of micro-needle arrays coded with pigments for one-time use. Each array represents exactly one code, as in Braille. The code is applied simply by putting manual pressure on all needles. The needles penetrate the skin of the animal, but only certain needles contain pigment, thus producing the code on the skin of the animal. The dot writing (data matrix code) can be read and decoded in a scanner.

Conclusions and recommendations

The working group strongly believes that it is important that the choice of identification method is based on scientific evidence rather than personal opinions and local traditions. Undoubtedly, more studies are needed to thoroughly evaluate identification methods and the introduction of a new method should be preceded by detailed scientific evaluation.

According to the Guide for the Care and Use of Laboratory Animals (1996) 'toe clipping as a method of identification should be used only when no other individual identification method is feasible and should be performed only on altricial neonates'.[34] When the Working group of Rodent identification began its work, distal phalanx removal was identified as a method with potential problems and therefore recommended as an area for further research.

Spangenberg *et al.*[11] initiated and performed one study and the study by Castelhano-Carlos *et al.*[20] was also performed after contact with members of the working group. In addition a third study has recently been published.[26] In summary, these recent studies have demonstrated that distal phalanx removal does not have any negative effects on growth and physical and behavioural development in young mouse pups.[11,20,26]

General recommendations:

- The ideal identification method should provide reliable individual identification, have no adverse effects on the animal or the animal model and be technically easy to apply;
- The choice of method depends on the age and size of the animal, whether or not a tissue sample is necessary, whether every animal needs a unique number, the duration of the study and whether the identification method can interfere with the research results or their interpretation;
- We recommend that proven permanent methods should be used for long-term identification and non-invasive temporary methods should be used when appropriate;
- Key points to consider while choosing an ID-method are summarized in Table 1.

If unique numbers are needed for every animal, tail tattooing, (non-metal) ear tags or microchips are the only methods possible. For long-term studies (> 3 months), tail tattooing is the safest method of these, since ear tags may be lost and microchips might induce tumours. Metal ear tags are the worst choice of an ID method because of the associated pain and distress as well as the risk of ear infections, tumours and allergic reactions. Moreover, subcutaneous ink injection is an invasive, painful but non-permanent method and is therefore not recommended as a method of identification.

All the new methods have advantages in terms of animal welfare and are recommended although their availability (and applicability) may still be limited. It is hoped that scientific studies will be conducted comparing these new methods with the most commonly used methods available today. So far, the microchip is the only method available supporting 'online' data transfer to a computer. It is notable that all four new identification methods described do provide a computer ID as well.

An important area for future research is the use of analgesia/anaesthesia during and after identification procedures. Inhalation anaesthesia does not in general alleviate post-operative pain. Therefore a special treatment for pain alleviation should be combined with this anaesthetic method. However, long-term pain is likely to have a more adverse effect on animal welfare than any acute pain experienced during and identification procedure. Local analgesia could be applied but this need to be administered in advance before it will have any effect. However, the body parts where it needs to be applied are very small and the animals can lick off any ointment used. Sometimes anaesthetics are aversive, which means that the degree of aversion from the anaesthetic could actually be greater than the transient pain of, e.g. ear notching (P Flecknell, personal communication). In addition, the restraining necessary for performing identification procedures has been shown to be as aversive to the animals as the identification procedure itself.[3]

In summary, the choice of identification method should be determined so as to minimize the adverse effects on the animals, while at the same time taking into consideration the type of research to which the animals will be subjected. There is no gold standard method because each situation is different (see above). Here too, good science and animal welfare go hand in hand.

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