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Mouse identification methods and potential welfare issues: a survey of current practice in the UK

NH Mazlan[1], N López-Salesansky[2], CC Burn[2] and DJ Wells[1]

[1]Department of Comparative Biomedical Sciences, The Royal Veterinary College, Royal College Street, London NW1 0TU, UK; [2]Department of Production and Population Health, The Royal Veterinary College, Hawkshead Lane, Herts AL9 7TA, UK

Summary

Marking mice to identify individuals is routine practice in laboratory animal facilities, but little is known about the current methods of choice or their perceived animal welfare, logistical or experimental design consequences. Therefore, an online survey on mouse identification was sent to laboratory animal establishments throughout the UK. The survey link was sent to 83 recipients, generating 62 responses from 54 animal establishments. Most establishments were academic (61%) and over 50% of the responses were from unit managers and/or named animal care and welfare officers. The two most commonly used identification methods were ear punch or ear notch (85%) and marker pen application (63%). The use of microchips had been discontinued by 20% of institutions. Toe clip, was considered to be severely stressful or/and painful by 53% of the respondent while microchips (45%) and tail tattoo (35%) were regarded as being moderately stressful or/and painful. Ear punch or ear notch was the most commonly used method for tissue collection for genotyping. Potential welfare issues associated with each identification method are discussed in the context of the survey results.

Keywords Mouse identification; laboratory animal welfare; refinement; standardisation; husbandry

Introduction

Most biomedical research is carried out on rodents, especially mice (mice were used in 71% of the 3.8 millions scientific procedures commenced in Great Britain in 2011).[1] Since mice are usually housed in visually homogenous groups, individual identification is often required. A wide range of methods have been used to identify individual mice, with some methods being more invasive in nature than the others. Permanent identification methods include ear notch, ear punch, ear tag, toe clip, tattoo and microchip. Temporary identification can be achieved by the use of hair dyes, fur trimming or non-water soluble marker pens. In general most permanent identification methods are

invasive (breaking the skin), while most non-permanent methods are usually non-invasive. Regardless of being invasive or non-invasive, all procedures involve restraint of the animal which is itself normally stressful,[2,3] although it may be possible to modulate the degree of anxiety and stress through the use of alternative handling and restraint methods.[4]

Identification marking schemes are rarely included in the Methods sections of scientific publications, but arguably they could be regarded as "Welfare-related assessments and interventions that were carried out before, during, or after [an] experiment", which are suggested for inclusion by the ARRIVE guidelines.[5] The invasive and/or intrusive nature of the methods means they have the potential to differentially affect mouse welfare, and are a possible source of variation that could affect experimental results.

Earlier in 2013, two working groups of the Federation of European Laboratory Animal Science Association (FELASA) have published separate reports on their survey findings and recommendations on animal identification and rodent genotyping. The FELASA Working group on animal identification found that ear notch/clip (20 out of 42 responses) and ear tag (15 out of 42 responses) were the most used methods in the USA/Canada and in Europe, while toe clip and ear tattoo were the least used methods. The group recommended using an identification method with minimal adverse effects on the animals while considering the type of research involved, although the precise methods of choice were not named.[6] From a survey covering 25 European countries, the FELASA working group on rodent genotyping found that tail biopsy (121 out of 158 respondents) was the most used method for sampling/genotyping, while ear punch/notch (72 out of 158 respondents) and ear tag (39 out of 158 respondents) were the methods of choice for identifying genetically modified rodents. That working group recommended using a method that is able to simultaneously identify an individual animal and provide tissue for genotyping.[7]

A literature review was carried out to find available information on mouse identification methods and their welfare consequences. The search terms were: rodent identification, mouse

identification, identification methods, marking methods, genotyping, microchip, transponder, toe clip, tattoo, ear tag, ear notch, ear punch and marker pen. The related references cited within the selected literature were also reviewed. A simplified overview of the advantages and disadvantages of each mouse identification and genotyping method are detailed in Table 1. In summary, most articles on different mouse identification and/or genotyping methods focused their investigation on the acute effects on mice, the ease of performing each method, reliability and durability of each method. Also, different institutions or even different researchers have their own set preference of mouse identification and genotyping method.

Table 1 ↑

There has been only limited investigation of the welfare consequences of each method for identifying mice. For example ear punching is a routine husbandry procedure but it is likely to cause stress during restraint, and because it penetrates the sensitive tissues, it may cause acute pain at the time of marking and potentially a degree of chronic pain afterwards. Indeed, mice vocalised more during ear-notching (30% of 26 mice) than a sham procedure (8% of 24 mice).[8] There is also evidence in other mammals; rats showed significantly greater mean arterial pressure during the period 1-16h after ear-notching than after micro-tattooing or ear-tattooing[9] while ear-tagged and ear-notched piglets showed increases in pain-related behaviours, vocalisations, salivary cortisol and blood lactate than controls.[10]

There is scope for refinement in marking methods. For example, in genetically modified mice, a biopsy is needed for genotyping, and it is possible to combine the biopsy with marking methods such as ear punch, ear notch or toe clip. Combining biopsy for genotyping with identification marking method would require only one potentially stressful event rather than two.[7]

Given the numbers of mice used in experimental procedures annually and the need for the majority of them to be unambiguously identified, there is potential for making significant welfare

improvements by choosing or modifying an identification method to minimise pain, stress and other negative welfare consequences. It is important to note that improvement to laboratory animal welfare will often not just benefit the animal (humane implication), but can also benefit the scientific community (scientific implication) by promoting valid, reliable and reproducible experimental data that are not being confounded by the element of pain and stress experienced by the animal.[11]

We conducted a survey on mouse identification to assess the current practice in the laboratory establishments throughout the UK, and perceived animal welfare, practical and scientific issues related to different identification methods. To our knowledge the survey is the most comprehensive to date (in terms of participation from one country), provides novel information summarising perceptions and practice in mouse identification in UK animal units.

Materials and methods

An online survey was created using SurveyGizmo (Online Survey Software & Questionnaire Tool) and the survey link was sent by e-mail to a mailing list targeting facility managers and Named Animal Care and Welfare Officers (NACWO) of laboratory animal establishments. Other personnel who are routinely involved in handling laboratory animals such as technical staff, scientists and Named Veterinary Surgeons (NVS) could also take part on behalf of the facility manager or the NACWO. Each response was anonymous unless the respondents chose to include their affiliation, so we made it clear that we only needed one response from each animal establishment for the survey results to be meaningful. We also promised to maintain the anonymity of individual institutions and individual respondents.

The survey comprised of 11 questions on mouse identification methods (Supplementary Material 1). Aside from straight forward questions on the current practice of identification and genotyping, there were also questions which required the respondents to rate stress or/and pain (three points from mild to severe) and level of ease (three points from quite hard and rather slow to

very easy and quick) associated with each identification method. Respondents were also asked to name the best identification method for experimental standardisation and rate the criteria of an ideal identification method (three points from being less important to very important).

The survey was carried out in two phases in the period of February to June 2012. Ethical approval for the survey was granted by the RVC Ethics and Welfare Committee (URN 2012 0052H).

Results

Survey coverage

We obtained 62 survey responses from 54 animal units from all over the UK: England (44 animal units), Northern Ireland (1 animal unit), Scotland (7 animal units) and Wales (2 animal units).

Background of respondents

Academic institutions made up the highest percentage (61%) of establishment type surveyed, followed by government scientific research institutions (GSRI) (17%), pharmaceutical establishments (13%), contract research organizations (CRO) and other types of establishments (4% each), while 1 respondent chose not to give any affiliation details (Figure 1a).

Figure 1 ↑

Most of the respondents taking part in the survey were unit managers and NACWOs, 55% and 48% out of the total number of 60 respondents, respectively (Figure 1b). Most of the time, the unit manager and the NACWO of a laboratory animal establishment were the same person (81% of NACWOs were also the unit manager).

Most respondents (67%) were between 35 and 54 years old (Figure 1c). Half of the respondents were females, 43% were males, while the remaining 7% chose not to include the gender information.

Mouse identification methods

A range of different mouse identification methods were used in laboratory animal establishments throughout the UK. The most commonly used methods were ear punch or ear notch (85%), marker pen (63%), microchip (31%) and ear tag (22%) (Figure 2a).

Figure 2 ↑

56% of the establishments had discontinued the use of some identification methods due to different reasons (described in Table 2). Among the methods that had been discontinued were microchip (37%), ear tag (30%), tattooing (23%) and toe clip (17%) (Figure 2b).

Table 2 ↑

Most mice were identified at the age of two to four weeks (61%) or between four to six weeks (15%) (Figure 2c).

Perception of potential animal suffering, personnel preference, level of ease associated with each identification method and preferred identification method for standardisation

The method perceived by respondents as causing the greatest harm to mouse welfare was toe clipping, with 53% of the respondents rating it as being severe, while 28% stated that they did not know about the degree of stress or/and pain of a toe clip procedure, and 19% rated the procedure as being moderately stressful or/and painful. 35% of the respondents regarded tail tattoo as causing moderate stress or/and pain, another 25% stated that they did not know about the degree of stress or/and pain, while 22% rated tail tattoo as being severely stressful or/and painful. The highest percentage of respondents regarded the microchip as being moderately stressful or/and painful (45%), while another 38% rated it as a mild procedure. Marker pen (82%), ear punch or ear notch (70%), hair dyes (63%), fur shave or fur cut (67%), and ear tag (41%), were rated by most respondents as being only mildly stressful or/and painful (Figure 3a).

144 Figure 3 ↑

145 Ear punch or ear notch had the highest percentage of respondents rating it as being most
146 preferred (57%), followed by microchip (34%) and marker pen (30%). The methods which most
147 respondents rated as being least preferred were toe clip (74%), ear tag (71%) and toe tattoo (64%)
148 (Figure 3b).

149 Identification methods rated as being very easy to carry out were marker pen (78%), hair
150 dyes (56%), fur shave or fur cut (55%) & ear punch or ear notch (52%). All tattooing methods were
151 regarded as being quite hard to carry out as 26% to 46% respondents gave this rating for each
152 tattooing method (Figure 3c).

153 A large percentage of the respondents listed microchip (76%) and ear punch (76%) as the
154 best identification methods for standardisation (Figure 3d).

155 **Criteria of an ideal identification method**

156 The criteria rated as being 'very important' by the most respondents were reliability (92% of
157 respondents), ease of reading the identification number or code achieved (89%), and having minimal
158 welfare concern (87%). Also, 75% of the respondents thought it was very important for an
159 identification method to be long lasting, and 71% of them thought ease of application was another
160 very important criterion for an ideal identification method (Figure 4).

161 Figure 4 ↑

162 **Genotyping**

163 The three most commonly used methods to collect DNA sample for genotyping genetically modified
164 mice in the UK were ear punch or ear notch (85%), tail snip (46%) and blood sampling (22%). Hair
165 pluck and toe clip were also used by 4% each of the animal units taking part in the survey (Figure 5a).

166 Figure 5 ↑

A high percentage (92%) of animal units practicing ear punch or toe clip to collect tissue samples for genotyping stated that they also utilized both methods for the purpose of identification (Figure 5b).

In the three cases where ear punch was not used to satisfy both purposes, respondents reported the following reasons; genotyping was only done on future breeding stock, or sometimes mice arrived already tagged or notched and researchers could not get genotyping results from ear notch sample obtained during identification so they performed a tail biopsy for genotyping.

When asked if they had found any disadvantages when attempting relatively non-invasive sampling procedures (hair pluck and mouth or rectum swab) to obtain DNA samples for genotyping, 12 out of 20 respondents reported that they found no disadvantages while the other eight reported they had found disadvantages. Five respondents gave details on the disadvantages as listed: 'hair pluck to collect DNA sample cannot serve as an identification method', 'hair pluck is still invasive to animal and easy to contaminate', 'some groups reported that their equipment was not sensitive enough to complete genotyping using samples obtained by non-invasive methods, or they are afraid of cross-contamination', 'hair sampling large number of mice resulted in contamination, and they still need to be identified' and 'mouth swab was not very good in giving clear genotyping results'.

Discussion

Looking at the survey results, it appears that some identification methods were more preferred by animal technicians or researchers than others. For example, ear punch or ear notch was used as an identification method in about 85% of participating animal units. Indeed, ear punch or ear notch is a quick procedure which requires only simple tools and therefore has lower running costs than other identification methods such as tattooing using a tattooing machine or implantation of a microchip.[12] Other methods such as fur shave or fur cut and tattooing were least used in the animal units surveyed. Fur shaving is not permanent[12, 13] while tattooing requires specific equipment and

191 sufficient training[6, 14] thus making them less favourable compared to other permanent identification
192 methods.

193 Ear punch or ear notch (which was the method of choice in most animal units) was rated as
194 a method which causes only mild stress or/and pain by 70% of respondents, putting it on a par with
195 other non-invasive identification methods such as fur shave or fur cut, hair dyes and marker pens.
196 This suggests that most people who work with mice assume that there is very little stress or/and
197 pain experienced by mice during ear punch or ear notch, despite some evidence suggesting the ear
198 punch is a potentially painful procedure as indicated by increased mean arterial pressure (in rats)[9]
199 and vocalisation[8]. This perception could be due to the fact that the procedure for ear punch or ear
200 notch is very quick with little opportunity for handlers to notice any sign of stress or/and pain.
201 Observing for the signs of stress or/and pain after returning mice to their home cage following the
202 procedure is not usually practiced and analgesia is not normally given. Further research may be
203 necessary to clarify whether or not ear punch or notch causes significant pain to mice.

204 Besides being non-permanent, non-invasive techniques could be the identification methods
205 that involve the least stress or/and pain. From the survey results, it was evident that marking using
206 marker pens, which were perceived by 82% of respondents to be a mild procedure, was practiced
207 widely (63%) in UK animal units. However, nothing is known about possible adverse effects of
208 marker pen inks for mice, which need to be investigated further, given that rats have been shown to
209 react in a complex manner. Tail-marked rats appeared bolder in an elevated plus maze, and yet they
210 showed more pronounced aversion-related Harderian gland secretion (chromodacryorrhoea) in
211 response to handling compared with unmarked cage mates; and (unmarked) rats avoided open pens
212 significantly more than closed pens in a choice test, suggesting that the solvent odour is aversive to
213 them.[15] There is also the possibility for toxicity or chemicals entering mouse's body which may
214 interfere with research results.[6]

Permanent identification methods such as toe clip and ear punch or ear notch will cause a variation in pain and stress levels due to variations in the handling duration, number of painful events (clips, punches and/or notches) and the amount of tissue being removed, according to their designated identification number. On the other hand, every animal may experience similar levels of pain and stress with other permanent identification methods such as ear tag and microchip. So, from this point of view, ear tag or microchip might be a more preferable permanent identification method for experimental standardisation. In agreement with the points mentioned above, the survey results showed that most respondents had chosen microchip (76%) and ear punch (76%) as the best identification methods for standardisation. The FELASA Working Group on animal identification considered metal ear tags (used by 22% of respondents here) as being the worst choice of identification method due to pain and distress as well as posing a risk for inducing various tissue reactions.[6] However, the literature has suggested that tissue reactions due to metal ear tags could arise from inaccurate placement of the tags or by using metal ear tags in a mouse strain known to be susceptible to squamous cancers.[16, 17]

Whenever tissue samples are needed for genotyping genetically modified mice, ear punch or ear notch would be the recommendation, as performing one invasive procedure to satisfy two goals is a refinement in experimental procedures,[7] unless less invasive procedures are possible, such as a mouth swab for genotyping and marker pen for identification, if these are indeed found to cause less stress. The FELASA working group on genotyping recommended ear punch or ear notch as the method of choice starting from 14 days of age, only when permanent identification and tissue for genotyping are needed.[7] Currently, ear punch or ear notch seems to be the method of choice for collecting tissue sample to genotype mice in the UK since 85% out of 54 animal units reported its use for genotyping. In fact, 90% of all units who perform ear punch or toe clip to genotype genetically modified mice reported that they utilize ear punch or toe clip as a mean of identification too. In comparison to our findings, a survey carried out by the FELASA working group has found that 46%

240 out of 149 respondents from 15 European countries including the UK reported using ear punch or
241 ear notch for genotyping genetically modified mice (weanlings or older).[7]

242 In our survey, we found that only two out of 54 animal units performed a toe clip for
243 genotyping purposes. There was not a single animal unit who reported the use of toe clipping for
244 mouse identification. By looking at questions in which we asked the respondents to rate the
245 procedure according to the level of stress or/and pain it causes, it was evident that respondents
246 regarded toe clip and all tattooing methods as causing a higher level of stress or/and pain than other
247 methods (Figure 3a). Relatively, they are also not easy to perform and would require a significant
248 training period before one can master the skill and gain sufficient experience. Although there are
249 articles reporting that three to seven days old mice showed little reaction to toe clip and that the
250 procedure did not significantly impair their grip strength, motor abilities, coordination and
251 balance,[14, 18] these results suggest that many do not regard the toe clip is as good as, or even better
252 than, an ear punch or ear notch.

253 Toe clipping is still a controversial, highly debated procedure in the UK. The
254 BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement recommended not to use toe
255 clipping, unless as an absolute last resort and that it should only be performed in mice below the age
256 of two weeks old.[13] On the other hand, Norecopa's (Norwegian Consensus Platform for
257 Replacement, Reduction and Refinement of animal experiments) Board has stated that toe clipping
258 should not be permitted even with the refinement described by the Norwegian Animal Research
259 Authority (allowing only one toe to be clipped on each hind leg).[19] However, in the latest edition of
260 the Guide for the Care and Use of Laboratory Animals by the National Research Council (USA), the
261 clause on toe clipping has changed from "*toe clipping as a method of identification should be used*
262 *only when no other individual identification method is feasible and should only be performed on*
263 *altricial neonates*"[20] to "*as a method of identification of small rodents, toe-clipping should be used*
264 *only when no other individual identification method is feasible. It may be the preferred method for*

neonatal mice up to 7 days of age as it appears to have few adverse effects on behaviour and well-being at this age (Castelhano-Carlos et al. 2010; Schaefer et al. 2010), especially if toe clipping and genotyping can be combined".[21] The change suggests that toe-clipping is now viewed in a different perspective after no scientific evidence of behavioural or motor impairment was found in two studies. In their recent publications, the FELASA Working Groups on animal identification and genotyping recommended distal phalanx removal (toe clip) for identification and genotyping in young pups approximately seven days old, by removing only the most distal phalanx of one toe per paw. [6,7]

The survey results demonstrated that there was a high level of welfare awareness among animal care personnel, as 87% of the respondents rated "minimal welfare concern" as a very important criterion of an ideal mouse identification method. Also, the use of several identification methods had been discontinued due to welfare concerns (Table 2). Ten respondents reported that they preferred to use other equally reliable identification methods that are more welfare friendly or less invasive in nature. Their concern for mouse welfare during identification gives a positive indication that they would be willing to improve their current practice if scientific evidence to support such a change on welfare grounds is presented.

There are certainly more questions that could have been added to the survey to make it more comprehensive, but at that point of time we felt that the questions were sufficient to establish the basic information on the current practice of animal identification in the UK. It would be useful to add a question on the number of mice kept in each facility and a few questions on the use of analgesic or anaesthetic during identification. Although there is a possibility of anaesthesia being aversive,[6] there is evidence that procedures such as ear tattooing in rabbits cause pain and application of EMLA cream prior to the procedure is effective in preventing pain associated with the procedure.[22] Furthermore, the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement recommended the application of local anaesthetic spray prior to tail tattooing.[13] Another plan for

future work is to target specific groups, such as animal unit staff, researchers and NVS, since the results from this survey comprised mostly of answers from Unit Managers and NACWO. Although some animal unit staff, NVS, scientists and a deputy facility manager took part in the survey, the number was fairly small and underrepresented, which makes a fair comparison impossible. In the future we would be interested to find out whether results from different groups would vary.

Since the survey, the Federation of European Laboratory Animal Science Associations (FELASA) Working Groups has published two reports with recommendations on rodent identification and genotyping.[6, 7] It should be noted that these reports may subsequently have influenced identification marking in the UK and elsewhere. Nevertheless, the survey has indeed given some useful baseline information on mouse identification methods used in the UK, and how they are perceived, particularly by Unit Managers and NACWOs. The welfare consequences of the commonly used identification methods have not been extensively studied, so further research is required to compare the most commonly used mouse identification methods, namely ear punch or ear notch, marker pen, microchip and ear tag.

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Table 1. Overview of the advantages and disadvantages of mouse identification and genotyping methods. Relevant references are given in superscript numerals where possible.

Methods	Advantages	Disadvantages
Ear punch/notch	1. Generally easy to perform, cost-efficient[10] 2. Easy to read, handling may not be necessary 3. Allows individual identification of maximally a few hundred mice 4. Provides tissue sample for genotyping[2, 9, 10, 11, 17]	1. Possibly painful[11] 2. Punched ear may induce aggression among cage mates[11] 3. Some strains reseal ear punches (eg. MRL/MpJ)[21, 22]
Toe clip/distal phalanx removal	1. Markings are truly permanent[17] 2. Provides tissue sample for genotyping[17] 3. Allows early genotyping – 3 to 7 day old pups[12, 16]	1. Possibly painful[17, 21] 2. Only to be done on mice before 14 days old[16, 17] 3. Handling or/and restraining may be needed to read markings
Ear tag	1. Allows identification of a very high number of individual mice[8, 10] 2. Quick & easy procedure[8] 3. Relatively inexpensive[8, 10]	1. Possibly painful[17] 2. Restraint may be necessary to read tag 3. May not be a permanent method – risk of losing tag[8, 10, 17] 4. Tag is a potential irritant[8, 10, 14-15, 23-24]
Tattoo • Revolving pliers (ear) • Lancet (tail & foot pad) • Micro tattoo system (ear/tail/ foot pad) • Electric tattoo equipment (tail)	1. Allows identification of a very high number of individual mice 2. Little risk of misidentification[17] 3. Footpad tattoo can be applied on mice of all ages[10, 12, 17]	1. Possibly painful[17] - in rats, micro tattoo more painful than others[6] 2. Personnel must be trained[8, 10, 12] 3. Anaesthetics or analgesics may be necessary[10] 4. Ink may fade/illegible with time[10]
Microchip	1. Allows identification of a very high number of individual mice[8] 2. Minimal identification errors compared to other methods[8, 10, 17,	1. Personnel must be trained for application & chip positioning 2. Expensive[8, 26, 27] 3. Potentially causes discomfort/ distress[12,26],

	3. Allow registration of data in computerized tracking systems[25, 26]	tumours[17, 27] 4. Handling or/and restraining needed to read chip-code
Fur shave/cut	1. Easy to apply (no special skills/training needed)[8] 2. Easy to read[8] 3. Less likely to be painful[8] 4. Low cost[8]	1. Temporary, may only last for 14 days[10] up to 3 weeks[11] 2. Need frequent handling to clip the hair[17] 3. Can only distinguish a limited number of mice[8] 4. Some shavers are noisy – possibly stressful to mice
Coat dyes/bleach	1. Easy to apply (no special skills/training needed) 2. Easy to read[8] 3. Less likely to be painful[8] 4. Low cost[8]	1. Temporary[10] 2. Need frequent handling to reapply dyes[17] 3. Can only distinguish a limited number of mice[8] 4. Potential toxicity[8, 11, 17]
Marker pen	1. Applicable to all ages[8] 2. Easy to apply (no special skills/training needed) 3. Easy to read[8] 4. Less likely to be painful[8] 5. Low cost[8]	1. Temporary, frequent remarking is necessary[10] 2. Potential adverse response to solvents in pens[8] 3. Aversive response to odour released from marker pen has been reported in rats[13]

Table 2. Reasons for discontinuing certain identification methods. Each reason was suggested by only one respondent, unless stated.

Identification method	Reasons for discontinuing
Microchip	Cost (n=4), microchips kept moving/slipping, loss of microchips, unnecessary, excessive for animal welfare, not needed anymore
Ear tag	Animal welfare (n=3), not easy to identify at a glance, difficult to identify after some time, can be torn off, tags fell out, more stressful to animal, front limb caught in ear tag and infection, very likely to tear off if males fight, used for specific reasons before
Tattoo	Welfare of animal (n=2), unnecessary for such painful method, too fiddly, other less invasive/equally reliable method available, caused local inflammation - deemed unsuitable for neonates by NACWO & NVS
Toe clip	Excessive for animal welfare (n=2), unnecessary, not visually easy to identify
Ear punch	Difficult to carry out and read, changed to microchip - linked to database, excessive for our needs and not easy to identify at a glance
Marker pen	Used only for short term study, now use mostly black mice, used for specific reasons before
Hair dye	Not permanent enough - frequent reapplication needed, took too long to apply, other equally reliable methods are available
Fur shave	Impractical
Bar code	Unreliable - attached using superglue to 1 day old pups, when they sweated the bar codes came off

Figure 1a. Type of institution taking part in the survey (n=54; GSRI, government scientific research institution; CRO, contract research organisation).

Figure 1b. Respondent's position (n=60; NACWO, named animal care and welfare officer; NVS, named veterinary surgeon).

Figure 1c. Respondent's age (n=60), with each slice labelled as the age category, followed by the number of respondents.

Figure 2a. Mouse identification methods used in UK animal units (n=54).

Figure 2b. Discontinued identification methods (n=30).

Figure 2c. Mouse age during identification (n=54), with each slice labelled as the age category, followed by the number of respondents.

Figure 3a. Identification methods according to animal stress or/and pain level as perceived by respondents.

Figure 3b. Identification methods according to respondent preference.

Figure 3c. Identification methods according to their ease of application. The number of responses for each method is given in Supplementary Material S2.

Figure 3d. Perceived best identification method for standardisation (n=62).

Figure 4. Criteria of an ideal identification method. The number of responses for each criterion is given in Supplementary Material 2.

Figure 5a. Tissue collection methods for genotyping (n=54).

Figure 5b. Using ear punch/toe clip for both identification & genotyping purpose (n=48), with each slice labelled as the method category, followed by the number of respondents.