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

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1 **Summary**

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

15 **Keywords** Mouse identification; laboratory animal welfare; refinement; standardisation;
16 husbandry

17

18 **Introduction**

19 Most biomedical research is carried out on rodents, especially mice (mice were used in 71% of the
20 3.8 millions scientific procedures commenced in Great Britain in 2011).¹ Since mice are usually
21 housed in visually homogenous groups, individual identification is often required. A wide range of
22 methods have been used to identify individual mice, with some methods being more invasive in
23 nature than the others. Permanent identification methods include ear notch, ear punch, ear tag, toe
24 clip, tattoo and microchip. Temporary identification can be achieved by the use of hair dyes, fur
25 trimming or non-water soluble marker pens. In general most permanent identification methods are

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

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

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

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

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

59 Table 1 ↑

60 There has been only limited investigation of the welfare consequences of each method for
61 identifying mice. For example ear punching is a routine husbandry procedure but it is likely to cause
62 stress during restraint, and because it penetrates the sensitive tissues, it may cause acute pain at the
63 time of marking and potentially a degree of chronic pain afterwards. Indeed, mice vocalised more
64 during ear-notching (30% of 26 mice) than a sham procedure (8% of 24 mice).⁸ There is also evidence
65 in other mammals; rats showed significantly greater mean arterial pressure during the period 1-16h
66 after ear-notching than after micro-tattooing or ear-tattooing⁹ while ear-tagged and ear-notched
67 piglets showed increases in pain-related behaviours, vocalisations, salivary cortisol and blood lactate
68 than controls.¹⁰

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

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

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

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

85 **Materials and methods**

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

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

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

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

104 **Results**

105 **Survey coverage**

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

108 **Background of respondents**

109 Academic institutions made up the highest percentage (61%) of establishment type surveyed,
110 followed by government scientific research institutions (GSRI) (17%), pharmaceutical establishments
111 (13%), contract research organizations (CRO) and other types of establishments (4% each), while 1
112 respondent chose not to give any affiliation details (Figure 1a).

113 **Figure 1 ↑**

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

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

121 **Mouse identification methods**

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

125 Figure 2 ↑

126 56% of the establishments had discontinued the use of some identification methods due to
127 different reasons (described in Table 2). Among the methods that had been discontinued were
128 microchip (37%), ear tag (30%), tattooing (23%) and toe clip (17%) (Figure 2b).

129 Table 2 ↑

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

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

134 The method perceived by respondents as causing the greatest harm to mouse welfare was toe
135 clipping, with 53% of the respondents rating it as being severe, while 28% stated that they did not
136 know about the degree of stress or/and pain of a toe clip procedure, and 19% rated the procedure
137 as being moderately stressful or/and painful. 35% of the respondents regarded tail tattoo as causing
138 moderate stress or/and pain, another 25% stated that they did not know about the degree of stress
139 or/and pain, while 22% rated tail tattoo as being severely stressful or/and painful. The highest
140 percentage of respondents regarded the microchip as being moderately stressful or/and painful
141 (45%), while another 38% rated it as a mild procedure. Marker pen (82%), ear punch or ear notch
142 (70%), hair dyes (63%), fur shave or fur cut (67%), and ear tag (41%), were rated by most
143 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 ↑

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

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

174 When asked if they had found any disadvantages when attempting relatively non-invasive
175 sampling procedures (hair pluck and mouth or rectum swab) to obtain DNA samples for genotyping,
176 12 out of 20 respondents reported that they found no disadvantages while the other eight reported
177 they had found disadvantages. Five respondents gave details on the disadvantages as listed: 'hair
178 pluck to collect DNA sample cannot serve as an identification method', 'hair pluck is still invasive to
179 animal and easy to contaminate', 'some groups reported that their equipment was not sensitive
180 enough to complete genotyping using samples obtained by non-invasive methods, or they are afraid
181 of cross-contamination', 'hair sampling large number of mice resulted in contamination, and they
182 still need to be identified' and 'mouth swab was not very good in giving clear genotyping results'.

183 **Discussion**

184 Looking at the survey results, it appears that some identification methods were more preferred by
185 animal technicians or researchers than others. For example, ear punch or ear notch was used as an
186 identification method in about 85% of participating animal units. Indeed, ear punch or ear notch is a
187 quick procedure which requires only simple tools and therefore has lower running costs than other
188 identification methods such as tattooing using a tattooing machine or implantation of a microchip.¹²
189 Other methods such as fur shave or fur cut and tattooing were least used in the animal units
190 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 vocalisation⁸. 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

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

229 Whenever tissue samples are needed for genotyping genetically modified mice, ear punch or
230 ear notch would be the recommendation, as performing one invasive procedure to satisfy two goals
231 is a refinement in experimental procedures,⁷ unless less invasive procedures are possible, such as a
232 mouth swab for genotyping and marker pen for identification, if these are indeed found to cause less
233 stress. The FELASA working group on genotyping recommended ear punch or ear notch as the
234 method of choice starting from 14 days of age, only when permanent identification and tissue for
235 genotyping are needed.⁷ Currently, ear punch or ear notch seems to be the method of choice for
236 collecting tissue sample to genotype mice in the UK since 85% out of 54 animal units reported its use
237 for genotyping. In fact, 90% of all units who perform ear punch or toe clip to genotype genetically
238 modified mice reported that they utilize ear punch or toe clip as a mean of identification too. In
239 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*

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

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

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

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

295 Since the survey, the Federation of European Laboratory Animal Science Associations
296 (FELASA) Working Groups has published two reports with recommendations on rodent identification
297 and genotyping.^{6, 7} It should be noted that these reports may subsequently have influenced
298 identification marking in the UK and elsewhere. Nevertheless, the survey has indeed given some
299 useful baseline information on mouse identification methods used in the UK, and how they are
300 perceived, particularly by Unit Managers and NACWOs. The welfare consequences of the commonly
301 used identification methods have not been extensively studied, so further research is required to
302 compare the most commonly used mouse identification methods, namely ear punch or ear notch,
303 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	<ol style="list-style-type: none"> 1. Generally easy to perform, cost-efficient¹⁰ 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} 	<ol style="list-style-type: none"> 1. Possibly painful¹¹ 2. Punched ear may induce aggression among cage mates¹¹ 3. Some strains reseal ear punches (eg. MRL/MpJ)^{21, 22}
Toe clip/distal phalanx removal	<ol style="list-style-type: none"> 1. Markings are truly permanent¹⁷ 2. Provides tissue sample for genotyping¹⁷ 3. Allows early genotyping – 3 to 7 day old pups^{12, 16} 	<ol style="list-style-type: none"> 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	<ol style="list-style-type: none"> 1. Allows identification of a very high number of individual mice^{8, 10} 2. Quick & easy procedure⁸ 3. Relatively inexpensive^{8, 10} 	<ol style="list-style-type: none"> 1. Possibly painful¹⁷ 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 <ul style="list-style-type: none"> • Revolving pliers (ear) • Lancet (tail & foot pad) • Micro tattoo system (ear/tail/foot pad) • Electric tattoo equipment (tail) 	<ol style="list-style-type: none"> 1. Allows identification of a very high number of individual mice 2. Little risk of misidentification¹⁷ 3. Footpad tattoo can be applied on mice of all ages^{10, 12, 17} 	<ol style="list-style-type: none"> 1. Possibly painful¹⁷ - in rats, micro tattoo more painful than others⁶ 2. Personnel must be trained^{8, 10, 12} 3. Anaesthetics or analgesics may be necessary¹⁰ 4. Ink may fade/illegible with time¹⁰
Microchip	<ol style="list-style-type: none"> 1. Allows identification of a very high number of individual mice⁸ 2. Minimal identification errors compared to other methods^{8, 10, 17,} 	<ol style="list-style-type: none"> 1. Personnel must be trained for application & chip positioning 2. Expensive^{8, 26, 27} 3. Potentially causes discomfort/ distress^{12, 26,}

	<ol style="list-style-type: none"> 3. Allow registration of data in computerized tracking systems^{25, 26} 	<ol style="list-style-type: none"> 4. Handling or/and restraining needed to read chip-code
Fur shave/cut	<ol style="list-style-type: none"> 1. Easy to apply (no special skills/training needed)⁸ 2. Easy to read⁸ 3. Less likely to be painful⁸ 4. Low cost⁸ 	<ol style="list-style-type: none"> 1. Temporary, may only last for 14 days¹⁰ up to 3 weeks¹¹ 2. Need frequent handling to clip the hair¹⁷ 3. Can only distinguish a limited number of mice⁸ 4. Some shavers are noisy – possibly stressful to mice
Coat dyes/bleach	<ol style="list-style-type: none"> 1. Easy to apply (no special skills/training needed) 2. Easy to read⁸ 3. Less likely to be painful⁸ 4. Low cost⁸ 	<ol style="list-style-type: none"> 1. Temporary¹⁰ 2. Need frequent handling to reapply dyes¹⁷ 3. Can only distinguish a limited number of mice⁸ 4. Potential toxicity^{8, 11, 17}
Marker pen	<ol style="list-style-type: none"> 1. Applicable to all ages⁸ 2. Easy to apply (no special skills/training needed) 3. Easy to read⁸ 4. Less likely to be painful⁸ 5. Low cost⁸ 	<ol style="list-style-type: none"> 1. Temporary, frequent remarking is necessary¹⁰ 2. Potential adverse response to solvents in pens⁸ 3. Aversive response to odour released from marker pen has been reported in rats¹³

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.