### Report of the 2010 RSPCA/UFAW Rodent Welfare Group meeting – the effects of husbandry on welfare and promoting good practice

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#### Introduction

The RSPCA/UFAW Rodent Welfare Group holds a oneday meeting every autumn so that its members can discuss current welfare research, exchange views on rodent welfare issues and share experiences of the implementation of the 3Rs of replacement, reduction and refinement with respect to rodent use. A key aim of the Group is to encourage people to think about the whole lifetime experience of laboratory rodents, ensuring that every potential negative impact on their wellbeing is reviewed and minimised.

The 2010 meeting focused on the effects of handling and cage change on rats and mice. Although both are extremely commonplace events in the lives of laboratory rodents, recent research suggests that they can have quite a profound impact on behaviour and welfare. The meeting took a closer look at the implications of these studies and discussed how people are handling and caring for rodents and what further refinements might be made. Two interactive discussion sessions focused on the implementation of animal welfare science and how this can be further encouraged in practice.

#### Handling and cage cleaning: effects on rat welfare

Charlotte Burn, Royal Veterinary College

Cage-cleaning and handling are the most direct interactions between laboratory rats and humans. Cage-cleaning is necessary for hygiene and health, but it could cause stress because it disrupts the animals' home environment and removes their scent marks and the important social information these provide. It also involves handling the animals and may expose them to increased noise and light levels, all of which can cause stress responses<sup>1</sup> (see Hurst below).

The optimum cage-cleaning interval should therefore strike a balance between maintaining good health and minimising stress to the animals. Intervals vary between establishments, usually ranging from every three to four days to weekly or every two weeks but it is not yet clear precisely how these different cleaning regimes affect the animals. Research on rat behaviour has shown that:

- cage-cleaning causes acute increases in activity levels but this does not necessarily indicate that the animals are stressed<sup>2</sup>;
- in adult male rats, cage-cleaning frequency shows no clear long-term effects on welfare<sup>3</sup>;
- adult rats show no preference for self-soiled cages over clean ones<sup>4</sup>.

This suggests that adult rats may cope relatively well with different frequencies of cage-cleaning. Refinements of the cleaning process itself, such as empathetic handling, could mean that stress is reduced, or even eliminated entirely; indeed, rats could potentially find some human contact enriching.

However, cage-cleaning might affect breeding rats more than non-breeders, because it often requires handling pregnant dams and the effects of disrupting the animals' environment are likely to be more significant if there are pups in the nest. This is because pups can become cooled<sup>5</sup>, the scent of the pups is removed from the nesting material and human or glove scents may be deposited onto the pups.

We carried out a study in which we compared cagecleaning in breeding Wistar rats at frequencies of twice a week, weekly and once every two weeks<sup>6</sup>. Parameters were monitored including female body weight, age at first reproduction, breeding parameters (litter size, number of litters per lifetime, numbers of pups born and weaned), pup weights and sexes, causes of any pup deaths and ammonia levels in the cages. Chromodacryorrhoea, or red staining around the nose, was used as an indicator of stress<sup>7</sup> (see also 2003 Rodent Meeting report<sup>8</sup>). Thirty-six pairs of rats were used per cleaning frequency and they were monitored for nine months.

We found that the different cleaning frequencies again had virtually no clear effects on any of the parameters used to assess rat health or welfare. The only exception was that breeding pairs were significantly more likely to cannibalise their pups if their cages were cleaned more frequently (twice weekly or weekly), particularly if cleaning occurred within two to three days of birth\*.

The reason for this is unknown, but one hypothesis is that cage-cleaning may have stimulated premature births. Births were more likely on cage-cleaning days (and this is thought not to have been an artefact of the recording system) and it is known that stress can trigger birth in humans and domestic animals<sup>9,10</sup>. Weak, dead or dying pups are more likely to be cannibalised<sup>11-13</sup> and pups born prematurely due to cage-cleaning may have been weaker and therefore more vulnerable to cannibalism. Alternatively, healthy pups may have been

killed and eaten by their parents (usually the males) if the removal of their odours caused the adults to perceive the pups as not their own, or if the parents sensed that cage-cleaning was a threat to the newborn litter. As cage-cleaning removes critical parent-offspring scents when pups are vulnerable and the litter becomes disrupted and chilled, either of these scenarios may have applied.

On this basis, it is possible to make some recommendations for refining cage-cleaning in breeding rats:

- Do not cage-clean during the last third of pregnancy or during the first three days following birth.
- Minimise noise and the transfer of odours between cages containing different individuals, e.g. by using cages with few noisy metallic components, cleaning cages in a ventilated area, or washing hands or gloves regularly.
- Transfer the whole nest to the clean cage if possible.

For non-breeding adults, current knowledge suggests that other aspects of rat husbandry are more important to rat health and welfare than the frequency of cleaning (although cage-cleaning frequency may affect some strains of rat more than the out-bred stocks used in studies to date). Non-breeding adult rats should therefore be cleaned as often as is necessary to maintain a healthy environment, for example keeping ammonia levels to acceptable limits. Finally, please note that all of the above applies to rats only – mice can be very different!

## Taming anxiety in laboratory mice by non-aversive handling

Jane L. Hurst, University of Liverpool

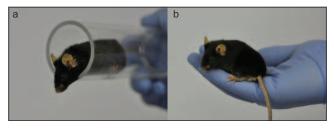
Handling laboratory animals is unavoidable, both for routine husbandry such as cage-cleaning and in order to carry out most scientific procedures. However, behavioural and physiological studies have shown that handling can be a major source of fear and stress in some species such as mice<sup>14</sup>. Although domestication has selected against the rapid flight responses typical of their wild ancestors, laboratory mice still seek to avoid capture and restraint unless they have learned that handling is not harmful.

Depending on the animal's experience, handling can have either positive or negative effects on stress responses, which can have an impact not only on welfare but also on the quality of scientific data. Surprisingly, though, little is known about the welfare implications of different methods for routine handling. In a recent study, we found that the method of handling is critical in determining whether handling induces fear and anxiety responses to human contact<sup>15</sup>.

<sup>\*</sup>A survey of delegates on the day found that 28% of their establishments routinely cleaned cages during the first two days post-partum and 59% did not.

Three techniques for picking up and handling mice were evaluated:

- tail handling the most common technique for picking up mice;
- home cage tunnel mice were guided into the home cage tunnel and lifted above the cage without direct contact (figure 1a);
- cupping on the open hand mice were scooped up on the open hands without direct restraint (Figure 1b).



**Figure 1.** Picking up mice using (a) their home cage tunnel and (b) cupping on the open hand.

Legend: In (b), inexperienced mice will immediately jump off the hand, but they can be trained to stay on by loosely closing the hands around them for up to 30 seconds on the first handling. Movies showing the handling methods and typical responses can be freely accessed at http://www.nature.com/nmeth/journal/v7/n10/abs/nmeth.1500.html

Behavioural studies compared the different handling methods. On the basis that handling techniques perceived as unpleasant by the mice would make them reluctant to interact with the handler, we observed the willingness of mice to voluntarily approach and contact the handler (or tunnel) before and after handling by one of the three methods. Stress during handling was quantified by scoring urination and defecation, as these are known to increase with anxiety and stress. We also used an elevated plus maze, which is a standard test of anxiety, to evaluate whether the different handling methods caused the mice to avoid open areas and show more "risk assessment" behaviours. For more detail on the experimental protocol and results, please see Hurst & West (2010)<sup>15</sup>.

We found that picking up mice by the tail induced aversion and high anxiety, whereas use of handing tunnels or scooping mice up on the open hand led to voluntary approach, low anxiety and acceptance of physical restraint (being held by the scruff and rotated onto their back). These responses were remarkably consistent across strains and sexes of laboratory mice, across handlers with different levels of prior experience, and across the light and dark phase of the diurnal cycle. The notable differences in response induced by these alternative methods for routine handling have not previously been recognised, maybe because picking up mice by the tail is so widely used in laboratories that the aversive and anxious response is perceived as 'normal'\*. More sympathetic handling techniques, that minimise anxiety responses, will enhance the welfare of the many millions of mice housed and handled in laboratories worldwide and will also reduce experimental confounds and provide more robust scientific outcomes. Changing handling techniques is one of the simplest (and cheapest) ways of improving welfare and staff satisfaction, as people can be reassured that they are improving the quality of life of the animals in their care. It can be easier to make the change by having a transition period in which the mice are picked up by tunnel for the first few days, then tipped onto the hand once they are used to being picked up and caught by hand or tunnel thereafter.

Recommendations for handling mice:

- Be aware that picking up by the tail induces aversion to handling and high anxiety the mice do not readily habituate to this.
- Catch mice using the home cage tunnel or open hands if possible, using a combination of the two for a transition period if this is easier.
- Avoid picking up by the tail unless anxiety is required, e.g. in behavioural experiments specifically studying anxiety.

# The effect of removing individual rats on indicators of welfare in the remaining group members

Oliver Burman, University of Lincoln

The social environment of a gregarious species such as the laboratory rat is likely to have a major influence upon welfare. From the individual rat's point of view, there are many positive aspects to group housing, such as a feeling of "safety in numbers" and opportunities for positive social interactions including allogrooming, huddling and play. There will also inevitably be some negative social interactions, e.g. agonistic behaviour or aggression.

The positive benefits of social housing should generally outweigh the negative aspects, but housing animals in captivity can affect their social environment. Particularly influential factors are the amount of space that is available, how resources such as food and water are provided, and group size and composition. Within the laboratory setting, social disruption can be caused by a number of factors including temporary or permanent changes to group composition (e.g. for randomisation), or permanent removal of individuals from social groups. The latter may be at weaning, or in order to maintain appropriate stocking densities in growing animals, or for experimental purposes.

Could this lead to welfare problems? Studies on other

<sup>\*</sup>Interestingly, our study found that lifting mice by the tail for abdominal inspection on the hand was not aversive – the aversion was induced by being *caught and picked up* by the tail.

species have found destabilisation of the social system following the removal of key individuals such as dominant males (in primates<sup>16</sup>) or no subsequent behavioural changes (in pigs<sup>17</sup>). In laboratory mice, removal of individuals (including dominant males) increased agonism among the remaining individuals<sup>18</sup>. It is therefore possible that standard husbandry procedures that disrupt the social environment, for instance via changes to the composition of individuals within a cage, may impact upon rat welfare compared to when the groups are left undisturbed.

We carried out a study to evaluate this, in which we investigated the effects of the permanent removal of individuals from social groups of young rats on behavioural and physiological indicators of welfare in the remaining animals<sup>19</sup>. The initial group sizes ranged from thirteen to twenty five rats (the study was part of a larger project investigating the effects of stocking density). Arbitrarily selected individuals were permanently removed on day seven and again on day fourteen. The impact on the welfare of the remaining rats was assessed using behaviour and faecal corticosterone metabolite levels, both of which were monitored both before and after the rats were removed.

We found that the remaining rats significantly increased agonistic behaviour, audible vocalisation, aggressive grooming, bar-chewing and climbing behaviour following removal of their cage-mates and that these behavioural changes were associated with a highly significant postremoval increase in their faecal corticosterone metabolite levels. The increases in bar-chewing and aggressive grooming, taken together, particularly suggest social conflict and stress in the remaining group members.

This may have been due to disruption of the dominance hierarchy or break-up of affiliative social relationships, although the groups were very large and had not been housed together long-term (e.g. since weaning). Alternatively, the responses could have been to the increase in space available to each animal resulting from the removals, where more active use of the space could have led to increased agonism – although there was no effect of removal on activity in general. For a full discussion, please see Burman et al. (2008)<sup>19</sup>.

Giving the animals the benefit of the doubt, the behavioural and physiological indicators of stress that were observed following removal of group members suggest the following recommendations:

- Leave social groups intact once established and avoid removing individuals if at all possible.
- When breeding animals, calculate stocking density on predicted end-weight.
- If removal is unavoidable, allow animals some control over their social environment increase

space, supply shelters and/or barriers to break up agonistic encounters (and provide escape routes), ensure there are sufficient enrichment resources for the entire group.

#### Looking in the wrong place ... implications for assessing pain and distress in animals

Matt Leach, Newcastle University

Considerable advances have been made in assessing pain in animals through the evaluation of behavioural and postural changes, for example following surgery<sup>20</sup>. However, successful assessment of pain and distress depends not only on establishing which *behaviours* indicate pain and distress but also on determining which *areas of the body* should be focused on to maximise the likelihood of observing these indicators. If an assessor knows to check for a particular behaviour but looks at the wrong body area, the effectiveness of behaviour-based assessment techniques will be reduced.

Recently we have used eye-tracking equipment to identify the observation patterns of experienced and inexperienced participants when asked to score video sequences of rabbits in varying degrees of pain (none, mild. moderate and severe) following ovariohysterectomy. These were all video clips of clinical cases taken from our video archive, where the ovariohysterectomy had been performed as part of routine husbandry practice and analgesia provided; no animals were operated on or denied analgesics for the purpose of this study. The video clips of rabbits in moderate to severe pain showed individuals where the analgesia used was insufficient to adequately alleviate the post-surgical pain. These animals received additional rescue analgesia directly after filming was completed.

We used 151 participants – drawn from animal technologists, veterinarians, scientists and lay people – of whom 71 had experience of caring for rabbits, while the rest did not. They were shown randomised video sequences of rabbits pre- and post-spay and asked to give each animal a pain score using a Visual Analogue Scale (VAS) that ranged from 0 (no pain) to 10 (most severe pain). The eye-tracking equipment was used to monitor each participant's gaze during the pain assessment, with the rabbit's body divided into different areas for analysis (Figure 2). We recorded how often the observers looked at each body part, how long their gaze remained on each body part and how long it took before the observer looked at each of the five body areas.

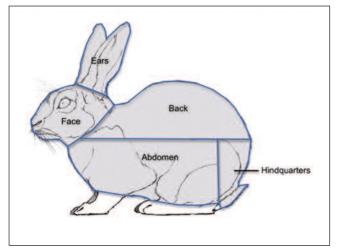


Figure 2. Rabbit body areas used in eye-tracking study

The results of our study demonstrated that irrespective of experience or gender, observers focused first, most frequently and for longest on the face compared to the abdomen, ears, back and hindquarters (P<0.001 for all comparisons). In addition, the ability of the observers to identify rabbits in pain was very poor and again was not affected by experience or gender. The ability to score pain correctly was positively correlated with increased observation of the back and hindquarters (which are the areas where pain-behaviours are observed following ovariohysterectomy in rabbits) but negatively correlated with observation of the face (P<0.05 for all correlations).

On the basis of these results, focussing on the face in order to assess abdominal pain using behavioural indicators is likely to be ineffective and increases the likelihood that important indicators will be missed. This suggests that people should be trained – and reminded – to focus on the most relevant body areas when assessing pain and distress in animals.

However, there is another way of looking at these results and their implications. If humans tend to fixate on the face, then identifying facial expressions in animals that are associated with pain could enable us to increase the effectiveness of pain assessment by incorporating animals' facial expressions.

A recent paper by Langford et al. (2010)<sup>21</sup> convincingly demonstrates that mice exhibit facial expressions associated with pain. The authors defined a Mouse Grimace Scale (MGS) that quantifies changes in five "Facial Action Units" (FAU); orbital tightening, nose bulge, ear position, cheek bulge and whisker change\*. Some of these are very similar to the changes that occur in humans experiencing pain.

We are currently undertaking a series of studies using our library of video clips of rodents and rabbits postsurgery, where the animals have already been assessed for signs of pain using behavioural scoring techniques. Participants including animal technologists, veterinarians and lay people are using still photos taken from the videos and scoring the FAUs according to the technique used by Langford et al. (2010)<sup>21</sup>. These studies are ongoing and preliminary results indicate that observers scored mice postvasectomy higher than pre-vasectomy animals, which is an encouraging result.

More research and evaluation of this concept is clearly necessary and it should be possible to achieve these benefits without causing additional harms, by using animals undergoing procedures that have been licensed as part of other scientific studies. In the light of current knowledge about the way in which people observe animals, these recommendations can be made:

- When observing animals, be mindful of where you are looking – do you know what the important indicators are and are you likely to see them? Think about the body areas you need to observe as well as the behaviours.
- Be aware that working with animals for a long time does not make anyone an expert observer in itself; awareness and empathy are needed too.
- Try looking at animals' faces when assessing welfare, keeping up to date with the literature for guidance.

#### A tasty alternative to the gavage needle for administration of retinoic acid

Stephanie Cadot, Androulla Economou and Mark Maconochie, University of Sussex

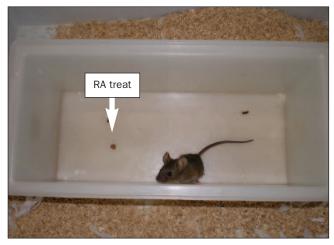
Retinoic acid (RA) is a derivative of vitamin A. It has several important functions as a signalling molecule in mammalian development, particularly in the developing anterior nervous system and inner ear<sup>22</sup>. Retinoic acid is also a major constituent of many medications used for the treatment of acne, dermatological inflammations and skin damage, and use of these products during pregnancy is not advised because of its potential to cause birth defects. RA is thus not only of considerable concern to fetal health in humans, but is also of interest for understanding normal embryonic development.

In experiments looking to understand the roles of RA in animals, it is routinely administered by oral gavage.

<sup>\*</sup>The MGS User Manual, produced by the paper's authors, includes pictures of all five FAUs and guidance on monitoring and scoring. This can be downloaded at http://tinyurl.com/5tm2doy

However, gavage does impose a welfare burden because of the stressful handling and restraint required<sup>23</sup>. In addition to this general distress involved in gavage dosing, there is a risk of trauma and damage to the oral cavity or oesophagus. In the worst case scenario, the gavage tube may be inserted into the trachea and the solution delivered into the lungs<sup>24</sup>.

We aimed to reduce stress in our mice, and minimise risks to their welfare, by investigating an alternative route for delivering RA by administration via voluntary ingestion of chocolate pellets. This required some limited training before the mice would reliably take the compound. The first "training treats" were made from chocolate spread (Tesco) mixed with icing sugar and water and shaped into pellets. Once the mice were accustomed to taking these, often after two to three training sessions, RA was added to the chocolate mix. Initially the pellets were too large, and the mice did not take the whole dose, but we were able to adjust the size and composition until the whole dose was reliably ingested. Mice are left unattended in a completely empty cage to eat the treat which also helps us to ensure that they have had a complete dose (Figure 3).



**Figure 3.** Feeding protocol for chocolate pellet administration of retinoic acid

Legend: The protocol can be modified to approximate normal practice in different animal units

In order to understand the molecular mechanisms underlying inner ear development, we have been examining the effects of RA on controlling the expression of the growth factor Fgf3, which plays an important role in the development of the ear<sup>25</sup>. Preliminary data demonstrates that RA-laced chocolate treats lead to extinction (loss) of Fgf3 expression. Moreover, the loss of expression is dose-dependent, indicating that the RA is reliably taken up and is fully bioavailable. Furthermore, significantly reduced doses of chocolate-RA doses relative to doses normally administered by gavage are able to elicit the same developmental effects, suggesting increased bioavailability through this new protocol.

This demonstration of *in vivo* effects of RA on Fgf3 expression suggests that administration of RA via chocolate treats may represent a realistic and viable alternative to gavage dosing of mice in this study. For further guidance on including substances in an animal's food or water, see the Joint Working Group on Refinement report on the administration of substances (Morton et al. 2001, pp 25-27<sup>24</sup>).

Recommendations for refinement of oral administration:

- Research the potential to administer the substance by mixing it into a palatable food or treat substance instead.
- Be prepared to experiment with the formulation and presentation of the substance – some training and patience may be required.

#### Practical implications: bringing animal welfare science to the cageside

Pascalle Van Loo, TNO, The Netherlands

Pascalle led a discussion session in which delegates shared their ideas and experiences of how to recognise and overcome obstacles and make use of opportunities, to enhance animal welfare in their own work environment. People considered successful and unsuccessful attempts to implement refinement and the main reasons why they did, or did not, succeed (Table 1).

Successful because	Unsuccessful because
Recognition that welfare was improved, in keeping with "Three Rs culture"	Financial constraints
Staff were prepared to implement refinement, including taking longer over husbandry procedures	It added time to the procedure
Support from researcher	Researcher was reluctant
Workload was reduced	Methods are established in the literature or perception that they are required for regulatory acceptance
Facility was prepared to spend the money	Lack of training in new methods
Support from Named Persons and/or Ethical Review Process	Full implications of changes were not thought through
Good communication and engagement between animal technologists and scientists; good support from all	Poor communication
Tenacity of animal technologists, even if this is just one especially committed person	Lack of backing from unit manager
Benefits such as increases in (or no change to) birth rate or reliability of data, or replacement of outdated methods	Difficult to quantify any improvements
Proof that equivalent refinement worked in other species	Insufficient knowledge of species-specific needs; lack of information in the literature

**Table 1.** Reasons for successful and unsuccessfulattempts at refinement

Legend: The reasons in the shades cells were mentioned most frequently by delegates

We concluded that animal welfare science has made huge progress in recent years and knowledge and understanding of what is good for animal welfare is ever increasing. However, attempts to implement scientific discoveries that could enhance animal welfare in a laboratory setting are unlikely to succeed unless animal technologists, researchers, animal welfare scientists and other relevant stakeholders work together and communicate effectively.

If refinement is to be successfully implemented, its effects on the welfare of the animal, on scientific validity and on the running of the animal facility and company or institute all need to be taken into account. This involves striking the optimal balance between animal welfare, scientific requirements and practicality.

From the animals' perspective, it is most important that any changes have genuine welfare benefits. For the scientific validity of results, it is most important that the intended refinement does not have a significant, negative effect on the science; or, even if results change in comparison to historical data, it should still be possible to draw valid conclusions. As demonstrated by some of the other presentations at the meeting, refinement can improve the science by reducing anxiety and stress, in which case it is the historical data that are likely to be less valid. For the animal facility, the most important factors to address are its culture, work load and staff job satisfaction, whereas for the institution as a whole, implementation costs of refinement versus benefits such as increased data quality and Corporate Social Responsibility (CSR) tend to be viewed as most important.

Recommendations towards the successful implementation of refinement:

- Make sure that all stakeholders are involved from the outset and throughout the implementation process. Depending on the establishment, type of study and particular refinement, this could include scientists, Named Persons, regulators, animal technologists and care staff, funding bodies, clients, company management and the ERP.
- Ensure that you make a serious effort to understand the issues and concerns raised by all stakeholders (and they should do the same).
- Highlight all of the potential advantages of the proposed refinement animal welfare, scientific, staff morale, CSR.
- Encourage all personal efforts to promote and implement refinement, however small.

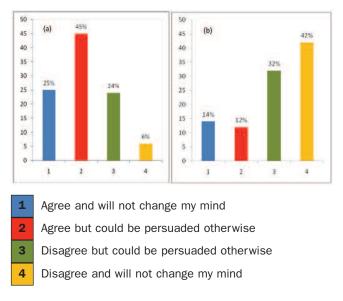
#### Summing up: Enforcement or Encouragement – how should we move good welfare forward?

Ngaire Dennison, Animals (Scientific Procedures) Inspectorate

Ngaire led an interactive session, using TurningPoint to collate delegates' views on the best way to facilitate

change with the aim of improving animal welfare in research and testing. Traditionally in many aspects of animal use, including the laboratory animal environment, minimum standards have been imposed as a method of ensuring the basic well-being of the animals involved, for example via Codes of Practice. This mechanism of enforcing minimum standards is one way to ensure that acceptable levels of care are implemented, but is it the only or best way to achieve higher standards of care and to ensure that good ideas for refinement are taken forward into daily practice? We focused the discussion on whether enforcement, or encouragement, was the best way to make progress, using the animal welfare science reported by the other speakers as examples.

This produced some interesting but not altogether unexpected, results. In the case of different handling techniques, delegates were asked the two questions illustrated in Figure 4.



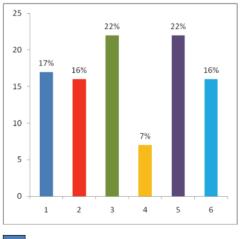
**Figure 4.** Delegates' views on welfare implications of capturing mice by the tail and whether capture methods should be enforced

Legend: Responses to the statements (a) "On welfare grounds, mice should not be captured using their tails; (b) "The Home Office should mandate that mice are not to be captured using their tails". Number of delegates = 120.

The majority of people (60%) accepted the researchers' findings and were prepared to agree that mice should not be captured by using their tails as a matter of principle (Figure 4a) but there was far less support for being forced to change handling techniques by the Home Office (Figure 4b). When this was discussed further, some people indicated that they were keen to find out about new developments in the understanding of animal behaviour, biology and welfare and implement these where they could but also felt that being mandated to do so by regulators would not take local issues and practicalities into account.

We also discussed how delegates obtained evidence to justify instigating new practices and what evidence they considered sufficiently robust to support making changes. How findings could be disseminated was a further topic for consideration. Support was strong for local initiatives in both of these areas. With respect to evaluating the effects of refinement, 37% of delegates felt that local studies provided enough evidence to justify implementation. The next highest votes were for an inter-laboratory validated study (16%), a withinlaboratory validated study (14%) and three or more published papers in refereed journals (13%). Delegates voted for welfare workshops and meetings as the most effective way to disseminate these findings (40%), followed by local training or other internal meetings (31%). Some people had only limited access to meetings, so were dependent on good internal communication about 3Rs improvements from colleagues who attended external events.

Following on from the discussions and surveys relating to the speakers' presentations and delegates' views on evidence gathering and dissemination, people were asked how they believed best practice could most effectively be adopted (Figure 5).



- 1 Local encouragement
- 2 Local enforcement
- 3 Industry/area encouragement
- 4 Industry/area enforcement
- 5 Regulatory encouragement
- 6 Regulatory enforcement

**Figure 5.** Delegates' views on the best way to ensure that best practice is adopted

Encouragement was preferred to enforcement in the case of both industry/research area and regulatory bodies, although there was no real preference for encouragement or enforcement locally. Encouragement from industry and regulators was viewed as the most effective driver towards best practice. There are both

positive and negative aspects to the use of encouragement and enforcement systems at whichever level they are applied, be it local, industry/area of research or regulatory, so it is likely that elements of each are needed to help to promote best practice.

The conclusions from this discussion, and the day's meeting, were:

- Good science is practised that is relevant to improving animal welfare in research and testing.
- Opinions differ on what is a sufficient evidence base to define good (and bad) practice.
- Information on refinement must reach all relevant people and stakeholders.
- Not everyone considers that they will be able to adopt best practice in all areas – there are different views on what reasons are acceptable for not doing so.
- Regulatory enforcement has a role, especially in setting *minimum* standards, but...
- Encouragement (with some level of "local" enforcement to back it up) was considered by the delegates to be the best way to ensure that good practice is adopted.

#### **Acknowledgements**

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