Report of the 2006 RSPCA/ UFAW Rodent Welfare Group meeting

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The RSPCA/UFAW Rodent Welfare Group holds a one-day meeting every autumn to discuss current welfare research and to exchange views on rodent welfare issues. A key aim of the group is to encourage people to think about the lifetime experience of laboratory rodents, ensuring that every potential influence on their well-being has been reviewed and refined. Speakers at the 2006 meeting presented preliminary findings of ongoing studies and discussed regulatory updates. Topics included the housing and husbandry of mice and rats, refining the use of rodents in asthma research, good practice for the euthanasia of rodents using carbon dioxide and achieving reduction by sharing genetically modified mice.

Speakers at the 2006 meeting of the RSPCA/UFAW (Royal Society for the Prevention of Cruelty to Animals/Universities Federation for Animal Welfare) Rodent Welfare Group presented several topics related to the 3Rs and laboratory rodents. Research presentations included the following: (i) effects of the frequency of cage cleaning on rodent welfare; (ii) effects of cage size and space allowance on different strains of mice; (iii) activity levels in two different mouse strains; (iv) a noninvasive method for monitoring lung function in rodent models of asthma; and (v) carbon dioxide as a means of euthanasia in rodents.

Speakers also presented updates on new legislation that will affect European standards for rodent housing and discussed initiatives for improving the sharing of genetically modified animals and for further developing 'mouse passports'. These passports are documents containing specific husbandry, welfare and genetic information that accompany mice when they are transferred between facilities.

RESEARCH

CAN WE IMPROVE THE WELFARE OF LABORATORY MICE BY CLEANING THEIR CAGES LESS FREQUENTLY?

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Cages must be cleaned regularly to limit the buildup of feces and urine (and subsequently ammonia) and to ensure sanitary living conditions. Cage cleaning, however, elicits numerous physiological and behavioral responses in laboratory mice that suggest that they find it aversive[1–7]. This is perhaps to be expected, given that free-living mice avoid areas that are frequently disturbed. Additionally, mice regularly deposit odor signals around their environment as a means of communication with other mice. There is a need to strike a balance between maintaining hygiene and minimizing disturbance to mice.

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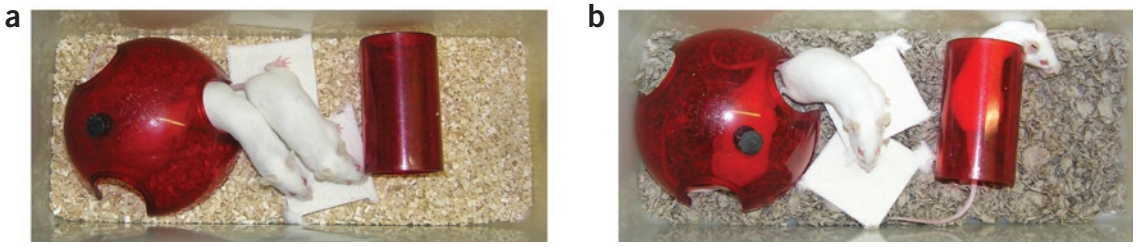

FIGURE 1 | Mice housed with Nestlets and a polycarbonate refuge and tunnel on (**a**) Aspen woodchip substrate or (**b**) TekFresh substrate.

We aimed to evaluate whether reducing the frequency of cage cleaning could improve the psychological well-being of mice without adversely affecting their health. We housed two strains of female mice (C57BL and BALB/c) using two types of substrate (Aspen woodchip; Lillico, Surrey, UK; and low-ammonia TekFresh bedding; Harlan, Oxon, UK). We provided mouse cages with Nestlets and a polycarbonate refuge and tunnel (Lillico, Surrey, UK; **Fig. 1**). We cleaned cages either weekly or once every two weeks.

We assessed physiological stress in mice by measuring corticosterone concentration in fecal samples. We also recorded the prevalence of stereotypic behavior (bar chewing) and evaluated mouse performance in a modified elevated plus maze task. We regularly checked ammonia concentrations in the cages. After six months we euthanized one mouse from each cage and assessed ammonia damage in respiratory tissues from its nasal tract, trachea and lungs. At the end of the study, we sent two mice per treatment from each unit for a full health screening by the Veterinary Services Department at Oxford University.

Preliminary results suggest that the frequency of cage cleaning did not affect the stress response in the mice studied. Though average concentrations of fecal corticosterone were lower in mice housed in cages that were cleaned once every two weeks, there was no statistically significant difference between groups in baseline concentration of fecal corticosterone or in 'peak' fecal corticosterone concentration 17 h after cleaning the cage. During the period after cleaning, mice in cages cleaned weekly tended to show more stereotypic behavior than mice in cages cleaned biweekly. Anxiety-related behavior in the elevated plus maze was more pronounced in BALB/c mice than in C57BL mice. It was also more pronounced in mice housed on Aspen substrate than in mice housed on low-ammonia substrate.

Researchers could smell ammonia strongly from cages that had not been cleaned in two weeks. When we examined the mice from these cages, however, we observed only mild to moderate respiratory tissue damage, indicating that the mice seemed to tolerate the ammonia concentrations in the cage. We recorded different concentrations of ammonia in various areas of the cage. The maximum concentration was 320 ppm (above the level of 25 ppm considered safe for humans[8]), recorded under the food hopper, whereas in the nest area ammonia concentration dropped to almost zero. Although the TekFresh substrate is marketed as 'low ammonia', after just one week ammonia concentrations in this substrate were the same as ammonia concentrations in Aspen substrate after two weeks. This may be because of the differences in the relative absorbency of the materials or in the ways mice used these materials, particularly in relation to their latrine.

In conclusion, though preliminary results were not statistically significant, there are some indications that cleaning cages once in two weeks rather than once a week can have certain positive effects on mice. Though the longer interval between cleaning may lead to increased ammonia concentrations and tissue damage, ammonia concentrations seem to remain within the range believed to be tolerated by mice.

EFFECTS OF CAGE SIZE AND SPACE ALLOWANCE ON THE WELFARE OF LABORATORY MICE

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The conditions in which laboratory animals are housed can result in physiological and behavioral changes that affect not only animal welfare but also the quality of research data.

We used a multidisciplinary approach to investigate the effects of cage size and space allowance on the welfare of laboratory mice. We used mice from inbred (C57Blk-6J) and outbred (ICR-CD1) strains. We housed mice in single-strain groups in cages of different sizes (floor space of 330 cm^2 or 960 cm^2) and with different allowances of floor space per mouse (60 cm^2, 100 cm^2 or 167 cm^2 for inbred mice; 100 cm^2 or 167 cm^2 for outbred mice). According to the Codes of Practice set out under the Animals (Scientific Procedures) Act 1986, we could not

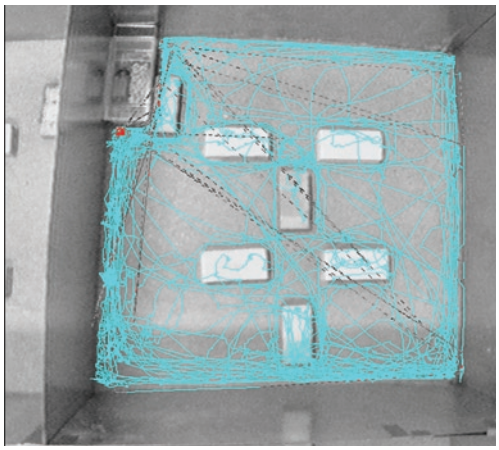

FIGURE 2 | Tracked movements of a mouse in an arena over a period of 2 h.

house outbred mice with a floor space allowance of 60 cm^2 per mouse because they were considerably larger than the inbred mice and grew heavier than 30 g during the study. We chose cage sizes after carrying out a survey of current practices and referring to the proposed new Council of Europe standards (discussed below in the section *Revisions of European legislation for rodent welfare*).

We used a factorial experimental design with six replicates for each of the ten treatments. We evaluated behavioral, developmental and physiological characteristics when mice were 6–11 weeks old and evaluated additional developmental, immunological and physiological characteristics post-mortem. We observed pronounced differences between strains in response to cage size and space allowance. This emphasizes the idea that different strains may be affected in different ways by the same housing environment. Specific results and their interpretation will be discussed in future publications.

MEASURED ACTIVITY LEVELS IN MOUSE STRAINS KNOWN FOR 'HIGH' OR 'LOW' ACTIVITY

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Mice of different strains may have varying behavioral or physiological requirements. The aim of our study was to examine behavioral and physiological differences between a standard, placid strain of laboratory mouse (LAC) and an outbred strain that is specific to our laboratory (PBI creams). PBI creams tend to be more active and reactive than other commonly used strains and seem to be more susceptible to the development of stereotypic behaviors. For these reasons, we predicted that when housed in small cages, mice from this strain might be more likely to show behavioral frustration than would their seemingly more placid counterparts (the LAC strain).

To investigate differences between the two strains, we used video recordings to assess the behavior of mice in their home cages and measured fecal corticosterone concentrations. We made all behavioral observations during the first 2 h of the dark phase, when mice are most active. To assess activity levels when mice were provided with more space, we placed mice in a large arena (1.4 m × 1.3 m), filmed them and later analyzed the distances they traveled (**Fig. 2**).

We expected that the PBI creams would travel further in the arena and would be more active in their home cages than would the LAC strain. Though the PBI creams did spend a significantly larger percentage of time performing ambulatory behaviors in their home cage, on average both strains traveled equal distances in the arena. In 2 h both strains traveled almost 150 m, the equivalent of circling around their home cage three times a minute. The PBI creams had significantly higher corticosterone concentrations than did the LAC strain. For both strains corticosterone concentrations were significantly higher in the arena than in the home cage. These preliminary results suggest that rather than highly active, the PBI creams are actually a highly anxious strain. The LAC strain seemed to cope better with being housed in a small enclosure.

In light of the observation that mice traveled substantial distances, it may be appropriate to consider providing some mice with running wheels in their cages. Running wheels are not equivalent to additional space, but some consider them to be a close substitute. Though there are concerns over development of stereotypies associated with running wheels, mice are highly motivated to gain access to them[9], and wheels may help reduce mouse anxiety in confined areas[10].

NONINVASIVE OBSERVATION OF LUNG FUNCTION IN RODENT MODELS OF ASTHMA

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We carried out two studies in two animal models of asthma (rats and guinea pigs), using a noninvasive method to record lung function. This method enabled reduction in the number of animals used and refinement of the experimental protocol.

We maintained conscious animals in a plethysmography chamber (Buxco, Ltd., Winchester, UK), in which they were free to move around, eat and drink (**Fig. 3**). Use of the chamber allowed us to measure pressure changes as an animal breathed, both under normal conditions and after administration of a substance that constricted its airways. The system's integrated computer software converted these pressure changes into airflow data and then derived

FIGURE 3 | A four-chamber plethysmography system.

from these waveforms a dimensionless parameter known as Penh. This parameter can be used as an indicator of airway constriction[11] and has been shown to correlate with changes in lung function[12,13].

Measuring the respiratory responses of sensitized (or allergic) rats to an inhaled allergen. In humans, exposure to allergens causes an early airway response (EAR) followed by a late airway response (LAR) with an associated airway inflammation[14–16]. Studies using invasive methods have demonstrated EAR and LAR in sensitized Brown-Norway rats that were anesthetized and challenged[17,18]. Antigen-driven changes in lung function and inflammation have been demonstrated in separate groups of animals[16,18].

We used plethysmography to evaluate the effects of potential anti-asthma therapies on EAR and LAR in conscious Brown-Norway rats. We used the same rats to measure clinically relevant parameters of lung function (such as peak expiratory flow) together with associated pulmonary inflammation, thereby increasing the amount of information obtained and halving the number of animals used. We have noted that the effects of inhaled steroid budesonide in this model correlate well with those observed in human asthma[19,20].

Comparison of the effects of different bronchodilators on mediator-induced bronchoconstriction in conscious guinea pigs. When an asthmatic is exposed to an allergen, the mast cells in the lungs degranulate, releasing mediators such as histamine and 5-hydroxytryptamine that act on smooth muscle, causing the airways to constrict. This study sought to determine the potency and duration of action of a new bronchodilating compound and to compare the compound with three bronchodilators already on the market[21].

Using traditional invasive methods, determining the potency and duration of action of four bronchodilators would require 20 treatment groups of 8 guinea pigs for each of 5 time points measured (a total of 800 guinea pigs). Because animals can be maintained under anesthesia for only a limited amount of time, groups of guinea pigs would be predosed with the test compound and anesthetized. Reactivity to a spasmogen would then be determined at set time intervals over 24 h (ref. 22). By using plethysmography, we were able to use the same guinea pigs at each time point. We therefore estimated that we reduced the number of guinea pigs used in this part of the study by one-fifth the number that would have been required using more traditional methods.

We also evaluated and compared the potential tachyphylaxis (decreasing response to a drug after repeated administration) of the test compounds. With traditional methods, two protocols would be required. In one protocol guinea pigs would be pretreated with a single dose of the test compound, and in the second they would receive a daily dose of test compound for 5 days. The use of plethysmography allowed us to obtain these data from the same guinea pigs that we used in the first part of the study, thus further reducing the number of animals required. No tachyphylaxis was observed in any of the compounds tested. These duration and efficacy data have subsequently been shown to correlate well with those demonstrated in humans[23].

We conclude that the use of plethysmography allows longitudinal studies to be carried out in the same animals with minimal discomfort, while enabling a substantial reduction in the number of animals used.

GOOD PRACTICE FOR CARBON DIOXIDE EUTHANASIA OF RODENTS

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Carbon dioxide is the most widely used euthanasia agent for laboratory rodents, yet debate continues over whether it causes pain or distress to animals. Exposure to high concentrations of carbon dioxide in humans causes both pain and distress[24], so there is good reason to examine whether carbon dioxide causes similar effects in animals. When animals are exposed to aversive concentrations of carbon dioxide, carbonic acid forms in the mucous membranes of the nose, mouth and corneal epithelium, activating nociceptors. Because the threshold for this activation is highly conserved across species and location, high levels of carbon dioxide may well cause pain to rats and mice.

This study used telemetry to investigate the time course of euthanasia using carbon dioxide in rats. Although carbon dioxide probably causes pain at concentrations higher than 50%, it is also a general anesthetic. If the carbon dioxide concentration rises gradually, rodents might lose consciousness before exposure becomes painful. Data from this study might therefore help to predict whether certain carbon dioxide administration methods (gradually raising carbon dioxide concentrations versus prefilled or rapidly filled chambers) are preferable for rodent welfare.

We euthanized adult male CD rats using carbon dioxide in a chamber (**Fig. 4**) that was either filled slowly (20% of chamber volume per minute), which is common practice in the UK, or prefilled (100% carbon dioxide). The prefill method is not permitted in the UK under Schedule 1 of the Animals (Scientific Procedures) Act 1986 but is commonly used elsewhere, including in the US.

We recorded brain activity (electroencephalogram, EEG), muscle response (electromyogram) and blood pressure in the rats using radiotelemetry transmitters (**Fig. 5**). When we placed rats in a chamber that was prefilled with carbon dioxide, brain activity was suppressed quite rapidly. The mean time to EEG silence was 39 ± 2 s, which is not much longer than that following decapitation (about 27 s)[25]. It is almost certain, however, that rats experienced pain for much of this period, as the carbon dioxide concentration was higher than the concentration that is presumed to cause pain during consciousness. Furthermore, bradycardia was observed while the rats remained conscious. This is likely to be a reflex mediated by the trigeminal nerve, which is provoked by irritant chemicals coming into contact with the nasal mucosa.

When rats were exposed to a slowly rising concentration of carbon dioxide, their behavior initially seemed normal, and brain and muscle activity were stable. After about 55 s, when total carbon dioxide concentration in the chamber was approximately 18%, the rats' movements became slightly uncoordinated (ataxia). The EEG then became more regular as the rats became anesthetized. After 110 s on average (29% carbon dioxide), no muscle activity or movement was observed. Loss of consciousness occurred after 156 s on average (39% carbon dioxide). As hypoxia set in, the EEG seemed to fluctuate once again, with a series of high-amplitude, very-low-frequency spikes related to brain hypoxia. The rats were deeply unconscious at this stage.

These preliminary results suggest that prefilling the euthanasia chamber ensures a rapid but probably painful death, whereas filling it slowly is not likely to cause pain, though it may cause distress by other mechanisms. For example, additional research has shown that carbon dioxide is highly aversive to rodents[26–28]. Dyspnea (the sensation of 'air hunger') may contribute to this aversion.

Despite recent attempts to achieve a consensus on the humaneness of carbon dioxide euthanasia[29], many questions remain to be answered. In the meantime, there are some practical measures that might immediately improve welfare. For example, a carbon dioxide diffuser can allow smoother distribution of carbon dioxide within the chamber compared with direct application of carbon dioxide through an inlet tube. This may reduce agitation during the process. Furthermore, it is preferable to introduce carbon dioxide gradually, rather than prefilling the euthanasia chamber. We recommend using an initial rising concentration of 20% of the chamber volume per min and increasing the flow rate once rats have lost consciousness.

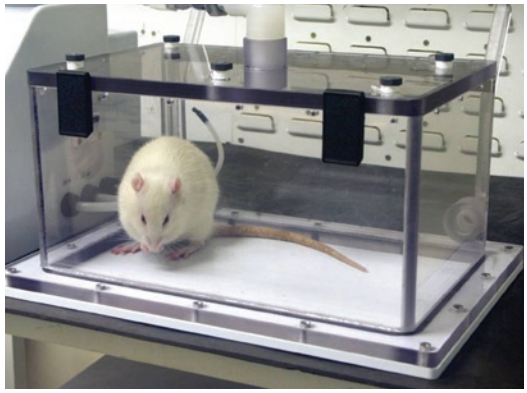

FIGURE 4 | Chamber used for carbon dioxide exposure. Gas is introduced through the tube at the top.

UPDATES

REVISIONS OF EUROPEAN LEGISLATION FOR RODENT WELFARE

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Legislation regulating the use of animals in research across the European Union is being revised. Part of this revision process has involved reviewing the minimum requirements for the housing and care of laboratory animals, currently set out in Appendix A of Council of Europe Convention ETS 123 (ref. 30).

In recent years our understanding of the requirements of animals has progressed, leading to general acceptance of the need for environmental complexity and social housing. Such provisions can result in improvements not only to animal welfare, but also to the quality and validity of scientific research.

Article 5 of Convention ETS 123 states that "any restriction on the extent to which an animal can satisfy its physiological and ethological needs shall be limited as far as practicable". With this in mind, the expert working groups set up by the Council of Europe set out to define animal housing protocols that met these criteria.

This presentation described the changes to Appendix A, as agreed in July 2006, with a focus on the new standards relevant to the housing and care of rodents. For example, the new Appendix recommends social housing as normal practice and requires specific scientific or veterinary justification for housing rodents singly. It also emphasizes the importance of providing appropriate bedding, refuge and nesting material.

In due course the UK Home Office will reissue Codes of Practice for the Housing and Care of Animals used for

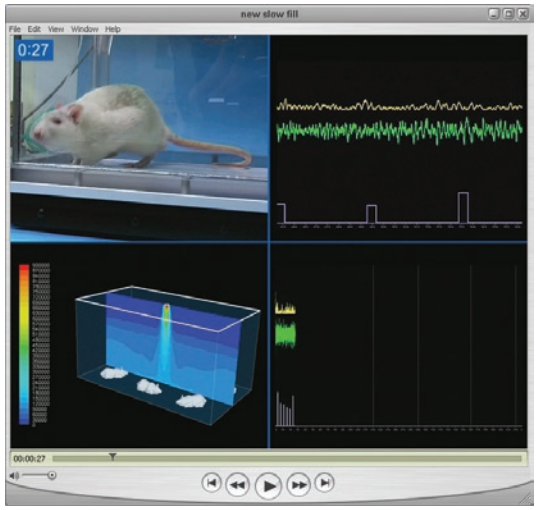

FIGURE 5 | Brain activity, muscle response and blood pressure were recorded in adult male rats to measure the time course of euthanasia by carbon dioxide.

experimental purposes, and these will include reference to provisions for rodents.

The Housing and Husbandry Sub-Group of the UK Animal Procedures Committee has produced a short document summarizing important differences between the revised Council of Europe standards and current UK recommendations. This will be useful for those building new facilities in the UK or refurbishing old ones. More information is available on the Animal Procedures Committee website (http://www.apc.gov.uk/).

RESOURCE SHARING AND THE FUTURE FOR 'MOUSE PASSPORTS'

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As more transgenic rodents are being generated, there is an urgent need to appropriately manage the sharing and archiving of these animals. Research Councils UK, a partnership of the UK's major biological and medical research councils, issued a position statement in 2006 regarding the importance of resource sharing[31]. In addition, several consultations regarding resource sharing took place in 2006, both within the UK and outside of it[32,33]. Like many aspects of scientific research, the sharing of resources is a global concern.

These recent developments highlight an increase in the number of international projects that require coordination of local participants. One such initiative is the Knockout Mouse Project, which aims to coordinate the efforts of labs in Europe, North America and Canada "to produce knockout alleles for all genes in the mouse genome" in order to provide researchers worldwide with "ready access to mice, their derivatives and data"[34]. During the course of this project some transgenic lines will in effect be remade. This repetition would not be necessary if lines were routinely archived and made available to the wider scientific community. Archiving lines and creating opportunities to share transgenic animals therefore constitute important refinements that can benefit animal welfare and reduce the number of animals used in research.

The RSPCA established a new Resource Sharing Working Group in 2006 to investigate the issues associated with sharing genetically modified mice within the UK scientific community. The group includes the Biotechnology and Biological Sciences Research Council, Medical Research Council and National Centre for the Replacement, Refinement and Reduction of Animals in Research. The group's initial aims include the following: (i) to identify current practices and to determine which archiving facilities and shared resources are presently available; (ii) to consider the potential influence that routine archiving and sharing may have on future research and the costs and benefits associated with it; (iii) to discuss what constitutes good practice; and (iv) to investigate ways of promoting archiving and sharing of genetically modified mice. The group is currently preparing guidelines on the sharing and archiving of genetically altered mice, which they plan to publish in 2008.

Considerations associated with archiving and resource sharing include identifying the best means of capturing the health and welfare information relevant to genetically modified mice, as well as ensuring the dissemination of this information between establishments. The UK Animal Procedures Committee has recommended that information that has welfare implications for the use of animals in biotechnology should be recorded and made available to any potential user[35]. The subsequent Genetically Altered Mouse Welfare Assessment Working Group took these recommendations a step further and initiated the idea of including this information in 'mouse passports', physical or electronic records that accompany mice when they are transferred between establishments[36]. Mouse passports currently take many forms around the world, and some consistency in their form and content might improve their impact on animal welfare. To this end, the RSPCA is convening a Mouse Passport Working Group. Additionally, it aims to organize a one-day meeting in the UK in 2008 to identify current practice and to discuss how best to take the mouse passport forward.

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COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

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