

## Collated Workshop Feedback – March 2021

### Improving the welfare of Mice used in the study of ageing

Human lifespan is increasing globally whereas healthspan - the period of life free from age-related diseases - is not increasing at the same rate. One consequence of this is that we are witnessing a concerted research effort into the causes of ageing and the control of its consequences, and this effort has resulted in an increase in the number of research groups that use ageing rodents – particularly mice – in their scientific studies.

This workshop aimed to explore the more common clinical signs of ageing and identify husbandry and care measures and humane endpoints. Groups also looked at two example protocols using mice aged for scientific purposes combined with the study of a disease process; the examples were for cancer and arthritis

### PLANNING, PREPARATION & CONSIDERATIONS FOR THE TEAM

The preparation and planning of the project is considered an important process. It is essential to have initial discussions to ensure everyone had the same picture/ idea of what is expected:

- Include technicians, vet(s), scientists (PPL holder and PIL holder(s) NIO and NTCO as appropriate
- Discuss baselines; weight, body condition and activity levels if possible. Ensure agreement around interpretation of endpoints, e.g. what constitutes the weight used as the baseline for any weight loss, agreement on body conditions score, etc. For arthritis study example, you would need to consider what baseline will be taken for gait and activity/ behaviour and consider how this can be measured
- Clarify (i) intervention points and (ii) endpoints
- Discuss how interventions and clinical signs accumulate in terms of severity – this should also be reflected in endpoints
- Training of personnel in the light of discussions, if required – to give consistency/ in any new techniques / monitoring practices
- Need to consider the impact of any mitigating steps on the scientific outcomes
- Separate score sheets for ageing and the signs associated with the inter-current disease of interest
- A considered approach so that animals are not (inadvertently) killed prior to completion of study for welfare issues associated with normal ageing
- Comparison can be made with an ageing animal without an inter-current disease
- This is a team process

## MITIGATIONS TO CONSIDER FOR AGEING

The below list immersed as new potential considerations to take into account when monitoring ageing animals

- Repeated anaesthesia an issue for older mice. Gaseous anaesthesia should always be used and there must be steps taken to keep the animals warm. Ensure prolonged body temperature maintenance
- Warmer ambient room temperatures for ageing animals, increase slowly week by week from 15 months to a minimum of 24°C (check with HO if going out with recommended)
- House ageing studies in a dedicated room – ideally at a quieter part of the animal unit
- Increased aggression may be minimised by improved/longer acclimatisation period
- Can enrichment items be used to encourage activity to prevent obesity and behavioural problems, e.g. access to running wheel, use of foraging, increased level of complexity in cage, larger floor area, more items for climbing?
- Ageing mice “bought in” have a high tendency to fight even if transported in an already established group, offer a larger area with enrichment and foraging material
- Have food pellets specially made that are of a smaller pellet size – doesn’t have the same problems of incisor teeth overgrowth that can be associated with softened foods and smaller pellets easier to handle by older mice
- Be aware of risk of tarsal joint injury (destabilisation of the joint and subsequent dislocation) this is particularly a risk with ageing males after 6 months and it can often be mistaken for a fight injury
- Visually muscle loss can be seen in ageing mice it is a continual, slow weight loss
- Cage-side monitoring for behavioural changes
- Use of automated behavioural monitoring of cage (especially in dark phase) to detect changes in behaviour
- Allow the animals to take control of their own environment, give males more space; if they are allowed to make their own hut from cardboard they will often create just one entry and exit point
- Cages with more height appeared to reduce aggression
- All listed on the common signs of ageing document (see below). In order of prevalence: Skin/hair; musculoskeletal; respiratory; cardiovascular; neurological/behavioural

## POINTS SPECIFIC TO THE ARTHRITIS MODEL

- ❖ Score sheet to assess the level of arthritis (multiple joints, degree of swelling) – however the current “standard” measures (in brackets) we NOT considered a good indicator of disease progression or animal welfare
- ❖ General welfare score (how “well” the animal is) – lack of grooming, etc.
- ❖ Evidence of pain – GRIMACE SCALE (not validated for this model and prone to issues with variability e.g. when just woken up)
- ❖ An endpoint BEFORE non-weight bearing should be identified

- ❖ Monitoring of gait/ activity/ behaviour - spontaneous versus evoked
- ❖ Could there be an “analgesic test” – so could animals suspected of arthritis receive dose(s) of analgesic to see if activity increased/ lameness decreased? Could this be used as an endpoint to demonstrate the presence of pathology?

## MITIGATIONS TO CONSIDER FOR ARTHRITIS

- Deep bedding/particle size of bedding
- Easy access to food and water with food on floor of cage (to limit the need for climbing)
- Pre-exposure to soft food to encourage use later if needed
- Analgesics: what, when, how much, how long? If not, why not?
- Discuss possible adjustments for aging animals within the team – technicians, vets or researchers with experience of the model or species may have ideas on possible solutions. Ask Named Information Officer to look at published information to see whether they can identify any other species-specific adjustments that could be made; e.g. “easier” ways to allow climbing
- Ideally monitor in cage via automated technology – decrease in activity likely to flag onset of arthritis

## POINTS SPECIFIC TO THE COLON CANCER MODEL

- ✚ The following 3 clinical signs will be impacted by ageing but not necessarily mean there is an imminent welfare issue: weight loss; body condition score; piloerection; and the animal may be considered as in “severe” when in fact it is just normal ageing
- ✚ Consider weight of tumour vs natural weight loss with ageing vs weight loss due to health issue. Tumour burden assessment should be done to help with this, visually the muscle loss can be seen in ageing mice and a continual, slow weight loss should be expected with ageing animals
- ✚ Monitoring of stool consistency
- ✚ Tumour burden assessment – Tumour may not be visible, so rapid weight gain or loss may be an indication of tumour growth
- ✚ Body scoring may be more useful for this model, especially abdominal distension

## MITIGATIONS TO CONSIDER FOR COLON CANCER

- ✚ Easy access to food and water
- ✚ More absorbent bedding

**“Common” signs of ageing in mice, grouped by organ/system** (reproduced from *Progressing the care, husbandry and management of ageing mice used in scientific studies*. Michael JA Wilkinson et Al)

Organ/System	Possible Clinical Signs/Pathology*
<b>Skin and hair</b>	<p><i>Hair thinning, hair loss, greying</i>                      Loss of vibrissae                      Dry, flaky skin; <i>unkempt coat</i>                      Delayed wound healing, greater propensity to post-op infections/abscessation                      Increased risk/incidence of cutaneous or subcutaneous tumours</p>
<b>Special senses</b>	<p><i>Ocular opacities, loss of vision, ocular or periocular infections</i>                      Dry, sunken eye/s  <i>Hearing loss</i>                      Loss of vibrissae</p>
<b>Cardiovascular</b>	<p><i>General slowing down; exercise intolerance</i>                      Increased risk/incidence of:                          Strokes (neurological signs; sudden death)                          Heart attacks/fatal arrhythmias (extreme collapse; sudden death)                          Poorer post-anaesthetic recovery/anaesthetic-related death</p>
<b>Respiratory</b>	<p>Nasal discharge, sneezing, coughing/chattering, rapid/shallow breathing, dyspnoea, aerophagia                      Increased risk/incidence of tumours</p>
<b>Digestive</b>	<p><i>Malocclusions, dental abscesses</i>                      Gastrointestinal dysfunction (diarrhoea; constipation; <i>changed Body Condition Score</i><sup>31</sup> [BCS])  <i>Rectal prolapse</i>                      Increased risk/incidence of tumours</p>
<b>Musculoskeletal</b>	<p>Arthritis/arthrosis/loss of muscle tone (swollen/painful joints; reluctance to move general slowing down; exercise intolerance; gait abnormalities; decreased grip strength)                      Hunched posture                      Increased risk/incidence of bone fractures/dislocations (pain)</p>
<b>Metabolic/endocrine</b>	<p><i>Less able to thermoregulate</i> (shivering; cold stress; piloerection; heat intolerance; decreased core body temperature)  <i>Less able to keep energy balance</i> (weight loss; weight gain)                      Less able to process/clear drugs (increased risk of toxicity)                      Increased risk/incidence of tumours</p>

