Standard Score Sheet for the assessment of wellbeing in mice: *NSG mice*

[Animal facility or facilities:]

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Project title:			
Animal Ethics #		Name of contact person:	
Chief investigator:		Contact number:	
Research Group:		After hours number:	

Scoring of wellbeing will be performed relative to the following assessment criteria:

Criteria	Score			
	0	1 (mild)	2 (moderate)	3 (severe)
Activity and responsiveness	Normal	Mild/slight reduction in activity relativdde to normal or previous observations	Isolated from cage-mates or obvious reduction in activity and responsiveness relative to normal or previous observations; alternatively, there is increased response to stimulus (e.g. appears agitated, twitching, easily started, or photophobic)	Stationary while awake for prolonged periods (>15min); when nudged, does not move, or makes poor attempts to move; collapsed and unable to right itself; persistent and prolonged fitting/ trembling (>1min)
Facial grimace (see image 1)	Not present	Facial grimace is subtle or inconsistent (i.e. only 1 to 2 of the "indicators" demonstrated in image 1 are moderately present)	Facial grimace is moderate (i.e. at least 3 of the 5 "indicators" are "moderately present")	Facial grimace is obvious (i.e. at least 3 of the 5 "indicators" are "obviously present") however, there must also be indication of other generalised symptoms (e.g. hunching, reduced activity)
Coat condition (see image 2)	Normal	Coat does not appear entirely smooth, clean and silky (i.e. the coat appears slightly 'rough')	Some indication of piloerection is present (i.e. 'rough' coat), but it is not obvious over the mouse's entire surface area	Generalised piloerection, i.e. obvious, very rough coat over majority of the body's surface area (as demonstrated in image 2)
Body position/ posture (see image 4)	Normal	Mild hunching (see image 3)	Moderate hunching	Severe hunching: or arching and writhing (despite analgesia having been provided)
Respiratory function	Normal	-	Increased respiratory effort (mild increases in respiratory rate, increased abdominal movement)	Increased respiratory effort compromising normal behaviours (i.e. the animal is lethargic, isolated and inappropriately responsive); or has slowed respiratory rate and gasping; or is open mouth breathing; or has blue mucous membranes or extremities; or noisy breathing (e.g. respiratory "clicking")
Tarsal swelling (see "Tarsal Swelling Score Matrix and image below)	All score 0	Score of 1 in any criteria and no score 2 or 3 in any criteria	Score of 2 in any criteria and no score 3 in any criteria	Score of 3 in any criteria
Body weight loss*	<5%	5-9% (relative to body weight recorded at the start of the experiment i.e. day 0)	10-14%	≥15%

*please note: body weight may not need to be measured at each monitoring point, particularly if monitoring is occurring frequently (as the handling associated with this procedure can be stressful for mice). In experimental mice that are asymptomatic, there is rarely reason to measure body weight more frequently than once a week. If disease symptoms present acutely body weight should be measured with appropriate frequency to appreciate impact to the animals (up to daily measurements). Where appropriate, body condition scoring (see image 5) may be used intermittently in place body weight measurements.

Cumulative Score:	**Action, relative to cumulative score:**
0	= no action (in addition to routine care and monitoring)
1 to 4	= symptoms observed, monitor at least daily (including all animals of similar treatment groups), if symptoms are unexpected seek veterinary advice
5 to 11	= monitor at least twice daily, provide food/water supplementation (e.g. wet mash or gel pack on the cage floor), if symptoms are unexpected seek veterinary advice
> 11	= euthanasia is required (unless otherwise advised by a facility veterinarian)

***** A score of 3 in any one category = euthanasia is required (unless otherwise advised by a facility veterinarian). If a veterinarian advises treatment that does not follow these criteria,** a brief veterinary report MUST be submitted to the AEC via the animal ethics veterinary officer: aeu.vet@uq.edu.au

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Specific actions relative to TARSAL SWELLING scores:

Management of animals at risk of developing tarsal swelling:

- NSG mice should be routinely housed on 'soft bedding' as apart of normal husbandry.
- Frequent handling should be avoided
- Handling should avoid placing the mouse on a hard surface like the cage top. Consider tunnels or cupping of mice. Forceful handling which places pressure on the tarsus should be avoided.
- All efforts should be made to handle these animals in "clean" manner. Strongly recommend handling animals only in the hood.
- For veterinary support, ensure contact is made with UQBR Veterinary Services <br.vetservices@uq.edu.au>

Tarsal swelling score and management matrix – see image below				
Score	0	1	2	3
Tarsal swelling – limb/s involved	Both hindlimbs are normal	One or both hindlimbs	One or both hindlimbs are	One or both hindlimbs
Redness and Swelling	None	Mild redness, inflammation and swelling	Moderate redness, inflammation and swelling OR Severe redness or swelling if NOT accompanied by any other severe signs	Severe redness, severe inflammation and swelling surrounding the tarsal joint if accompanied by a 3 in any other criteria
Gait	Normal	Reduction in limb movement	Observable reduction in movement or limp present.	Limb movement severely affected to non-weight bearing.
Retraction of limbs during restraint	None	Mild retraction during restraint.	Moderate retraction of the limb/s during restraint.	Severe retraction of the limb/s during restraint.
Toe grip	Normal	Mild loss of toe grip	Toe grip is moderately affected.	Toe grip is absent
Ulceration	None	None	Some mild ulceration	Moderate to severe ulceration
Monitoring	As approved	At least daily	Twice daily	Twice daily
Total Tarsal score	**0** All score 0	**1** Score of 1 in any criteria and no score 2 or 3 in any criteria	**2** Score of 2 in any criteria and no score 3 in any criteria	**3** Score of 3 in any criteria
Management	No change	Local treatment of tarsus, change in bedding	Local treatment of tarsal area, change in bedding	Euthanasia OR veterinary supervised multimodal analgesia and submission of a brief vet report
Analgesia*	None	May not be needed	Should be considered	MUST be given

*Unless previously approved in AEC application, all analgesics are to be prescribed by veterinarian.

NOTE: Approval is given based on mice being euthanised when reaching a score of 3 for tarsal swelling. If veterinary-supervised treatment is undertaken for any mice that have a score of 3 in any tarsal swelling criteria, a brief veterinary report MUST be submitted to the AEC via the animal ethics veterinary officer: aeu.vet@uq.edu.au

Local treatment of tarsus: Apply F10 skin prep or chlorohexidine formulation e.g. Bepanthen antiseptic or antibiotic ointment once daily or as advised by attending veterinarian.

Analgesia: Unless previously approved in AEC application, analgesia must be given under veterinary advice. Examples of analgesia include: paracetamol, opioids (buprenorphine, tramadol), NSAIDs (carprofen, meloxicam). For severe pain, multimodal analgesia should be considered (i.e. using combinations of analgesics). Preparations of these drugs may be administered orally (ad lib) and/or via injection.

Analgesia combinations to avoid: 2x NSAIDs, administered together, as this will result in serious risks of side effects without benefit to analgesia e.g. meloxicam cannot be administered concurrently with carprofen. Note: paracetamol is not considered a "true" NSAID, and so it may be used concurrently with NSAIDs.

Ad lib oral analgesia is generally the preferred route of administration for these animals, given it limits handling requirements (especially in long-term studies).

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Preliminary Phenotyping

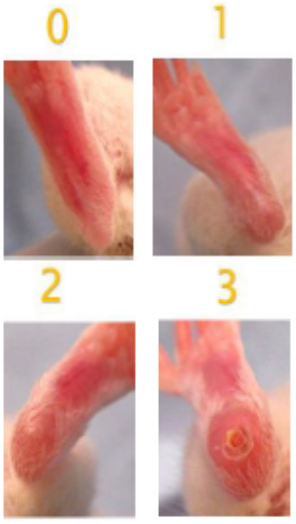

Figure 1: A clinical scoring system was developed to track the progression and severity of these lesions. This has previously been shown to directly correlate to pathology scores.

Clinical Scoring System	
0	Normal Limb; Heel is more pointed
1	Mild Lesion; Very early signs of heel lesion characterized by mild inflammation and widening of the calcaneal region. A limp may be present and retraction of affected limb during restraint may occur as well. Ability to use toes may be affected.
2	Moderate Lesion; Moderate inflammation and rounding of heel. Retraction of affected limb occurs during restraint and a limp is present. Ability to use toes is most likely affected.
3	Severe Lesion; Severe inflammation/swelling and ulcer/scab formation. Gait severely affected and retraction of limb occurs during restraint. Unable to use toes to grasp.

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Image 1. The 5 "indicators" of facial grimace, as per Mouse Grimace Scale: https://www.nc3rs.org.uk/grimacescales
Please note: facial grimace can be subtle and requires experience to be able to assess accurately. If you are not comfortable assessing this parameter, you must seek training and support for this purpose – contact your Chief Investigator and the relevant animal facility staff.

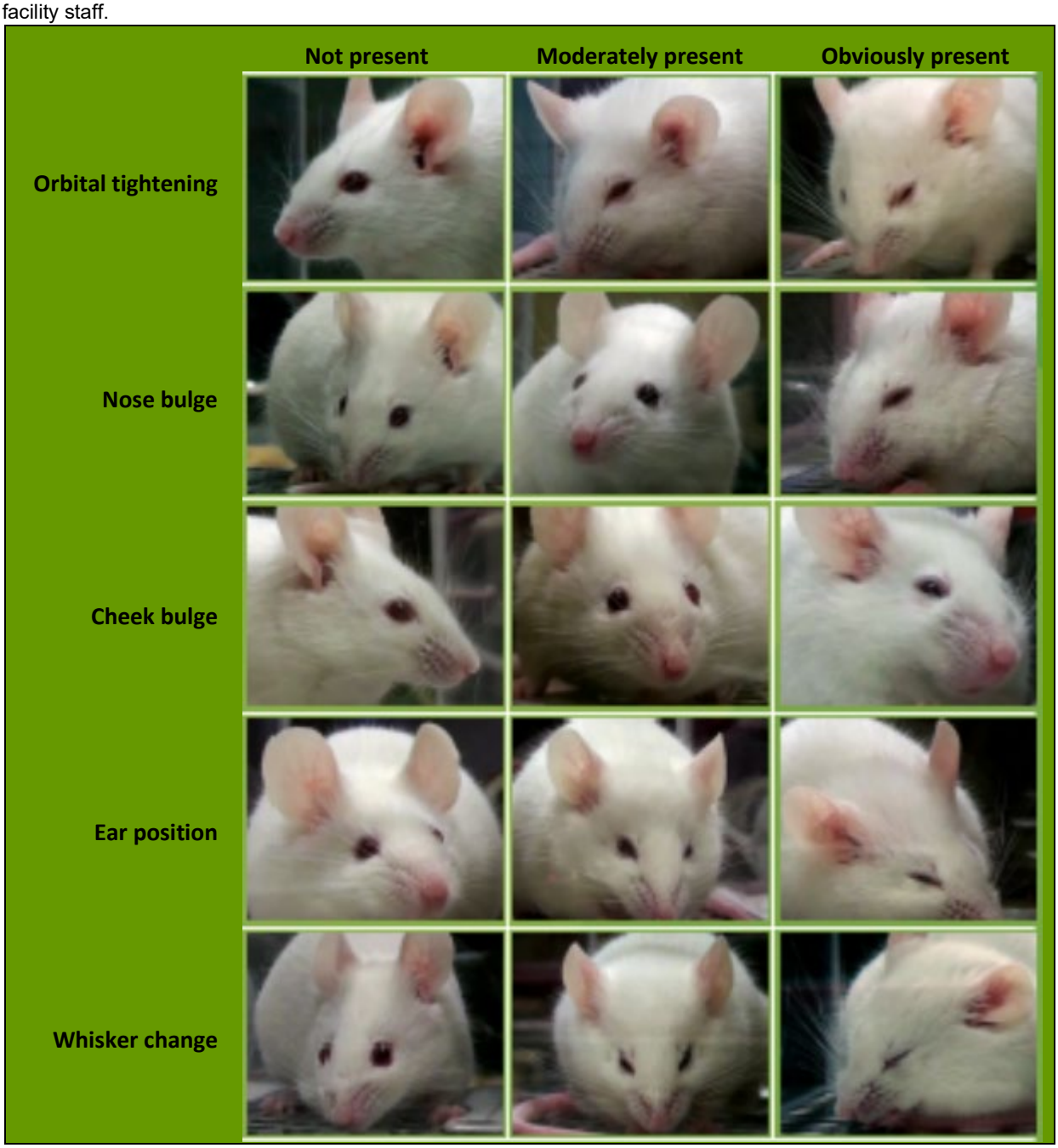

Image 2. Score 3 (severe) for the criteria: "coat condition". This mouse has diffuse piloerection, image source: https://www.humane-endpoints.info/en

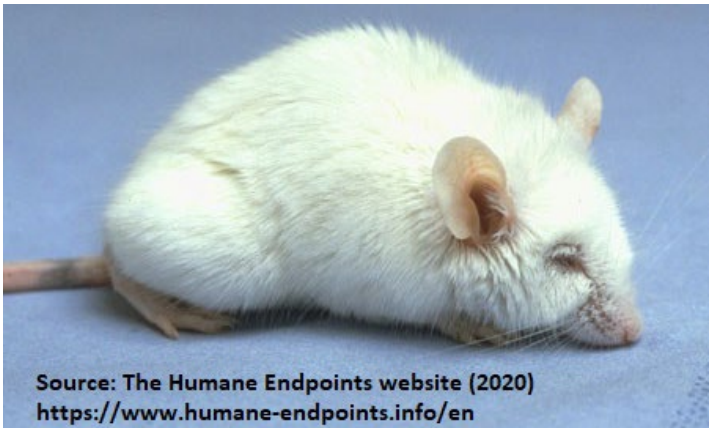

Image 3. Clinical scoring system for severity of tarsal lesions/swelling in immunocompromised strains of mice, image source: Campagna & Hernandez et al. Evaluation of Tarsal Lesions in Immunocompromised Mouse Strains, The Jackson Laboratory)

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Image 4. Hunching, scored from normal (score 0) to severe (score 3), modified from: Sevcik MA, Jonas BM, Lindsay TH, et al. Endogenous opioids inhibit early-stage pancreatic pain in a mouse model of pancreatic cancer. *Gastroenterology*. 2006;131(3):900–910.

Please note: ear tag-identifiers (as displayed in this image) are generally not considered appropriate for use in mice, as there are other, more refined methods of individual identification.

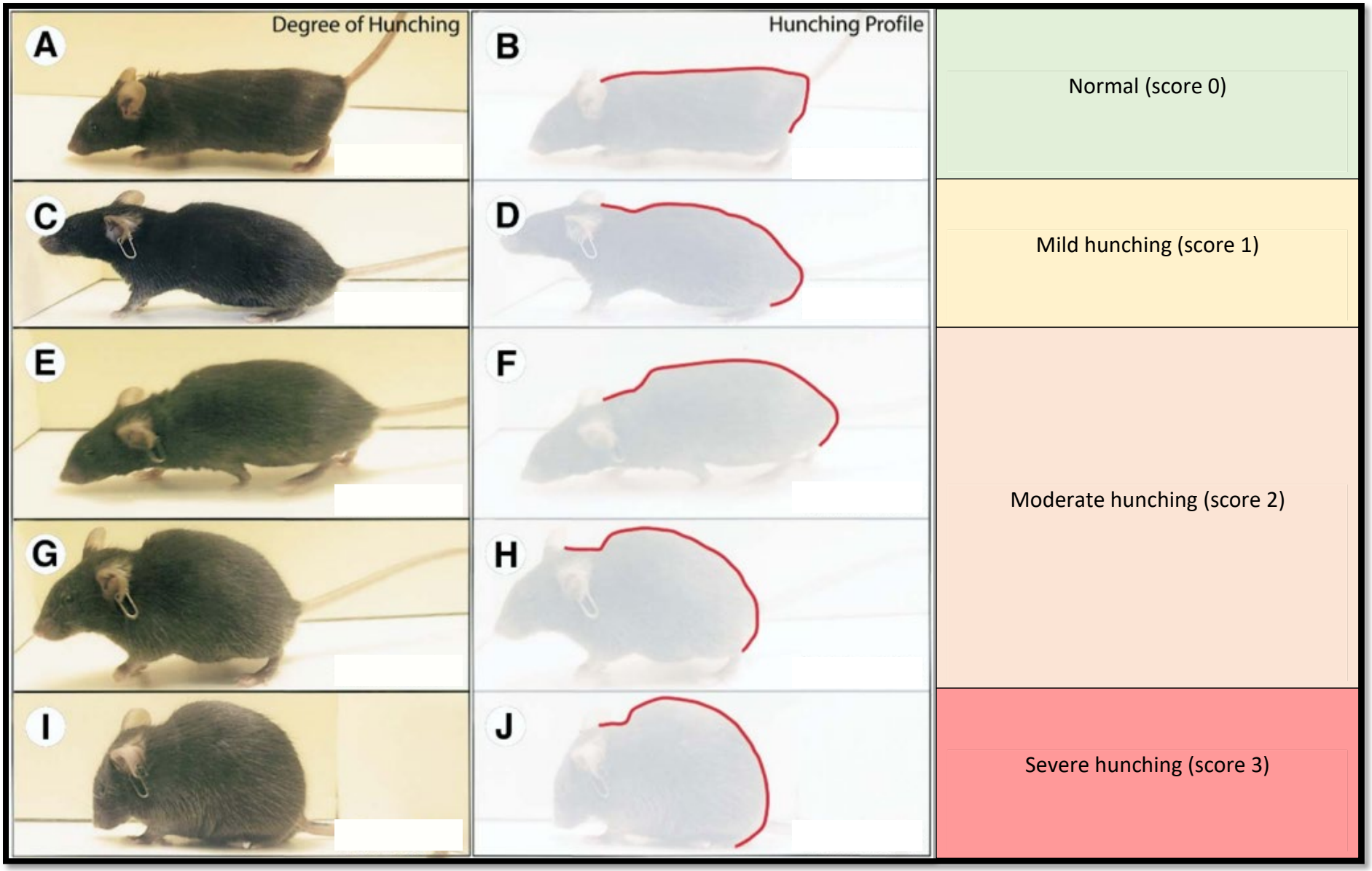

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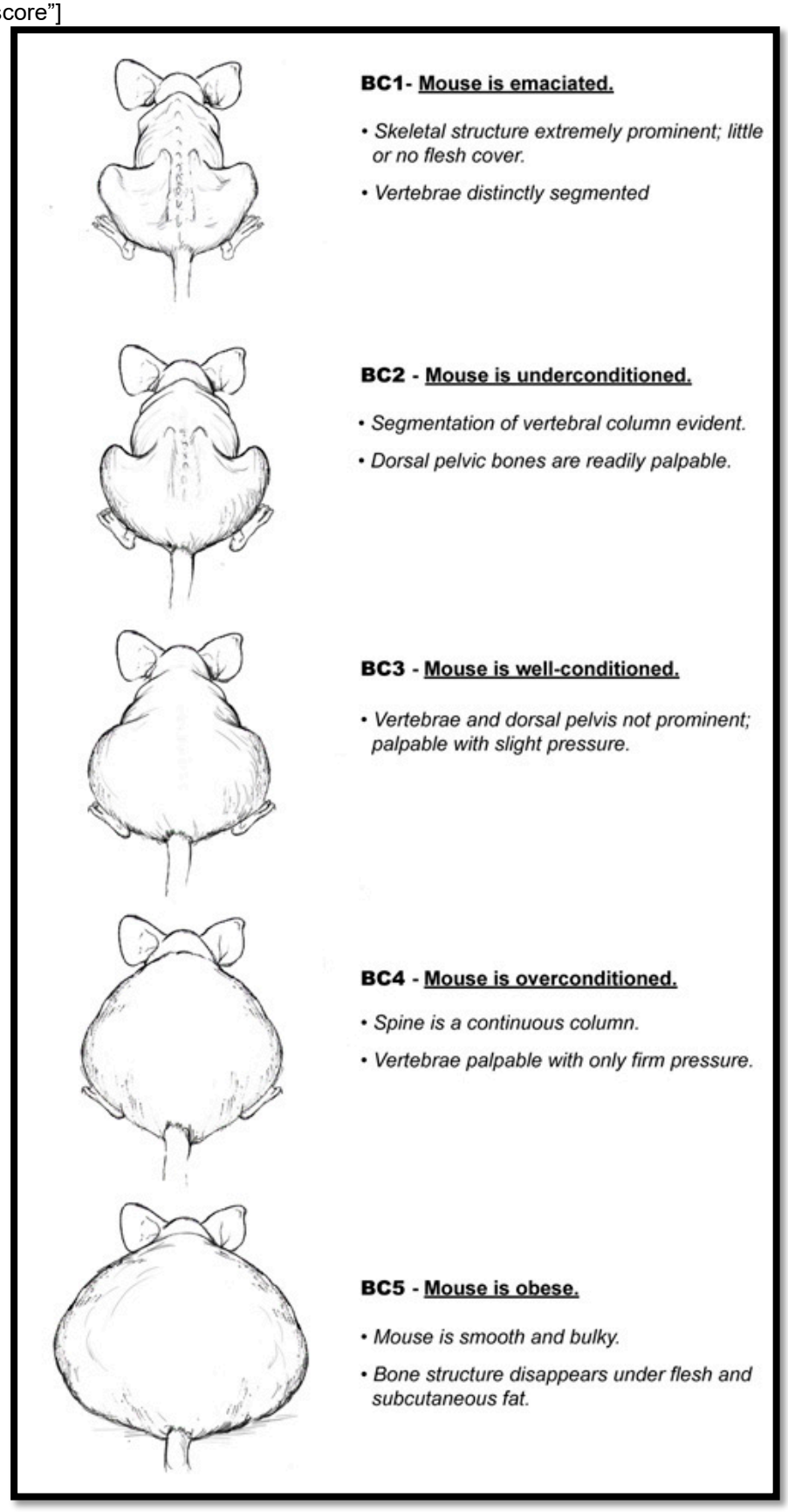
Image 5: Mouse, Body Condition Score reference.

"Body Condition Scoring (BC) is a quick, easy and reliable method for assessing mouse health. It utilizes a scoring system of 1 to 5 with 3 being the optimal condition, 1 being emaciated and 5 being obese." Source: Burkholder T, Foltz C, Karlsson E, Linton CG, Smith JM. Health Evaluation of Experimental Laboratory Mice. *Curr Protoc Mouse Biol.* 2012;2:145–165. [Body condition scores should be used with this score sheet "Standard Score Sheet for the assessment of wellbeing in mice" to avoid excessive handling (through repeated weighing) when performing frequent monitoring. Body condition scores complement body weight measurements; however, their use does not contribute to the score sheet's "cumulative score"]



Monitoring record for the assessment of wellbeing in mice

(see "Standard Score Sheet… NSG mice" for a description of the scores relative to the criteria below)

Project title: **AEC approval #:**

Chief Investigator: **Name of contact person:** **Contact number:** **After hours:**

Experimental/ Treatment Group: **Mouse ID: _____**

Date																													
Day (of study)																													
Criteria (scores 0 to 3):																													
Activity & responsiveness																													
Facial grimace																													
Coat condition																													
Body position/ posture																													
Respiratory function																													
Tarsal swelling																													
Body weight loss*																													
Body weight change (%)																													
Body weight (g)																													
Cumulative score (i.e. total)																													
Comments: (please use a key)																													

*Please note: body weight may not need to be measured at each monitoring point. Refer to "Standard Score Sheet… NSG mice" for details.

Cumulative score: **Action, relative to cumulative score:**

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> 11 = euthanasia is required (unless otherwise advised by a facility veterinarian)

***** A score of 3 in any one category = euthanasia is required (unless otherwise advised by a facility veterinarian) *****

Original mouse weight (g)		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Weight loss (%)	5%	15.2	16.2	17.1	18.1	19.0	20.0	20.9	21.9	22.8	23.8	24.7	25.7	26.6	27.6	28.5
	10%	14.4	15.3	16.2	17.1	18.0	18.9	19.8	20.7	21.6	22.5	23.4	24.3	25.2	26.1	27.0
	15%	13.6	14.5	15.3	16.2	17.0	17.9	18.7	19.6	20.4	21.3	22.1	23.0	23.8	24.7	25.5

Monitoring record for the assessment of wellbeing in mice

(see "Standard Score Sheet… NSG mice" for a description of the scores relative to the criteria below)

Project title: **Name of contact person:**
Chief Investigator: **AEC approval #:** **Contact number:**
Date: _____ **Day of study: _____** **After hours:**

		Criteria (scores 0 to 3):										
Mouse ID#	Treatment group	Activity & responsiveness	Facial grimace	Coat condition	Body position / posture	Respiratory function	Tarsal swelling	Body weight loss*	Body weight change (%)	Body weight (g)	Cumulative score (i.e. total)	Comments:

*Note: body weight may not need to be measured at each monitoring point. Refer to "Standard Score Sheet… NSG mice" for details.

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	10%	14.4	15.3	16.2	17.1	18.0	18.9	19.8	20.7	21.6	22.5	23.4	24.3	25.2	26.1	27.0
	15%	13.6	14.5	15.3	16.2	17.0	17.9	18.7	19.6	20.4	21.3	22.1	23.0	23.8	24.7	25.5