October 17, 2017

Dr. Shane Renwick,
Manager, National Issues and Animal Welfare,
Canadian Veterinary Medical Association
339 Booth Street,
Ottawa, ON
K1R 7K1

Re.: Draft CCAC Guidelines: Mouse

Dear Dr. Renwick:

The draft Canadian Council on Animal Care (CCAC) Guidelines on Mice (Guidelines) were reviewed in consultation with Drs. Michelle Groleau, Patricia Alderson and Jacinda Flood, Laboratory Animal Medicine veterinary colleagues.

The draft Guidelines: Mice (Guidelines) successfully present an enormously complex and voluminous subject matter in a relatively accessible and succinct format. Indeed, if this is a general guidance document on mouse animal welfare in the scientific context, it does elucidate current thinking and approaches to murine animal welfare and how to best approach this. It is a welcome replacement to the current ‘outdated’ Guidelines. The CCAC is to be commended.

Excellence in murine animal care and welfare requires an alignment of resources and efforts between research granting agencies, research stakeholders and individuals involved in the daily use and care of mice. Necessarily this requires an allocation of not inconsiderable academic and administrative financial, research and human resources together with alignment with industrial (commercial) partners in the animal, animal services, equipment, materials (food, bedding, other) and software supply chains. Indeed, where industrial partners (e.g., caging suppliers) do not produce equipment, systems or sundry goods compatible with the Guidelines standards as revised, this will necessitate workarounds with consequences for institutional research productivity and infrastructure (e.g., financial, material and human resource; physical resources: mouse housing capacity; care-associated labour).
Accordingly, flexibility in the interpretation and application of the *Guidelines* by the CCAC is essential to align these important and sometimes competing interests. The good news about these *Guidelines*, with some exceptions, is that they are generally an articulation of current vivarial, animal health and animal care committee practices. Specific issues are addressed in the attachment and are offered to the Canadian Veterinary Medical Association (CVMA) and CCAC as suggestions for refinement of the mouse Guidelines document.

Should you have any questions, please do not hesitate to contact us.

Sincerely,

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cc. M. Groleau, P. Alderson, J. Flood
APPENDIX

Issues

A) 2.4 Cage Size: As institutions acquire new cages, they MUST at least meet the minimum standards for floor area and height.

- This is predicated on caging availability from industrial partners.
- Canada represents a small market share for laboratory animal cage and equipment producers which are US and European-based and focussed.
- In the table of recommended spaces allowances, the minimum space allowance example for minimum enclosure size should read: **2-3 adult mice can be kept in the minimum enclosure size** rather than 1-3 as single housing runs contrary to optimal mouse welfare.

B) 2.4.2 Height – Guideline 5: Cages should be at least 13 cm in height...climb on the bars of the lid...

- The reference points for 13 cm are required. Some cages have tops that indent into the cage. Is there an allowance for integrated food hoppers? For example, Allentown durable cage bottoms measure at 13cm from the side, but when wire bar lid is in place, it takes about 1.5 cm off height of cage, and in the area of the hopper, even more so.
- Some commercial disposable caging systems do not have wire bars. Would the recommendation then be to supply a climbing apparatus of some sort?

C) 2.5 Cage Design: The design of the cage should allow for daily observation of the animals without disturbance...fear of open spaces...

- Practically, this is hard to do with the required amount of nesting materials and the recommended hiding areas or huts.
- Indeed, daily observation without disturbance can’t be done if mice are not in an open space where they can be visualized. Substitute parameters should be acceptable such nest building, food consumption if mice are not visually accessible.
D) 2.7 Cage Materials: Cages must be made of materials that are easy to clean, non-toxic, non-absorbable, durable, and resistant to heat and chemicals, and also be escape and predator proof.

- A definition or description of durability would be helpful.
- Insert the qualifier ‘cleaning’ in front of ‘chemicals’ unless the reference to chemicals extends to and includes experimental chemicals.
- A brief discussion of disposable caging systems should be included under this heading.

E) 2.9 Feeders and Water Supply: Mice should have access to clean food and clean water at all times. Food and water should be supplied in such a manner that they are easily accessible for all animals and contamination is minimized.

- ‘Food’ should be qualified by the statement life stage appropriate food.
- The appropriate quality of water for health and experimentation.
- The appropriate quality of water for health and study at all times.

F) Core Facilities for Generation of Genetically Modified Mice: …Core facilities usually include facilities for cryopreservation...

- Cryopreservation can be outsourced and mention of this should be made in this section.

G) Item 3.1.1 – Lighting: The 325 lux, 1 metre from the floor may be too high but depends on housing conditions.

- Retrofitting vivarial lighting may be problematic (complexity; costs; human health and safety, etc.). The CCAC must give due consideration to alternative strategies to address lighting intensity welfare concerns.
- Albino animals should be specifically mentioned under this heading as an exemplar of the unique needs of some mouse strains.

H) 3.1.2 – Temperature and Relative Humidity: Room relative humidity of 40 to 70% with an acceptable range of 30 to 70% is acceptable for mice.

- While mice may be able to tolerate these ranges in humidity, what is the recommendation?
- HVAC systems may not be able to provide high levels of humidity particularly during winter months.
Consideration must be given to the tolerance of room finishes, equipment and materials with high humidity.

Consideration must be given to human health and safety in high ambient humidity environments.

High cage humidity can contribute to food and bedding moulding as well as increased bedding bacterial concentrations and ammonia levels. Humidity effects at the cage micro-environmental level may necessitate increased cage change and handling frequency which runs contrary to murine animal welfare, where frequent handling is discouraged.

I) 3.1.3 Air Quality and Ventilation: As noted in the CCAC guidelines on: laboratory animal facilities-characteristics, design and development (CCAC, 2003), “the rate of air exchange within a room must be such that clean, fresh air is available to all animals and personnel at all times.” See Besch (1980) for factors contributing to good air quality...

- A general statement should be included regarding mouse cage stocking density and strain-specific needs. Indeed, it may be appropriate to insert this comment as an overarching statement.

J) 3.2 Personnel – Guideline 8: Mice should be monitored daily by trained personnel with minimal disruption to the mice.

- Monitoring parameters should be outlined (e.g. animal behavior, nest building, other) unless these are discretionary and within the purview of the veterinary team and animal care committee.

K) 4.3 Standard Operating Procedures – Guideline 10: Standard Operating Procedures should be in place for procedures and tasks related to shipping and receiving mice to ensure that the acquisition and transportation procedures protect the welfare of the animals...These SOPs should be applicable to all mice in science, including those that are procured as feed for other animals, such as for wildlife species and reptiles.

- The word ‘feed’ should be qualified by the word ‘live’ in order to distinguish these feeder mice from pre-deceased commercially purchased mice...which are not within the CCAC’s jurisdiction, other than in the broader biosecurity context.

L) 4.4.2 Moving Mice Between Institutions – Guideline 12: Prior to shipping, mice must be assessed to be fit for transport.

- Fitness for transport is relevant also to moving mice within an institution (see 4.4.3) and a statement to this effect should be included under this heading.
Guideline 13: Personal vehicles or public transport should not be used to transport mice.

- The separation of section 4.3.3 allows for an interpretation that personal vehicles could be used for intra-institutional animal transport. Clarification is required.

M) 4.6 Procurement of Mice for Feed: If mice are to be procured as feed for maintaining other animals (e.g., wildlife or reptiles), they should be acquired from a reputable supplier (as defined in the CCAC guidelines on: procurement of animals used in science (CCAC, 2007)) to avoid bringing diseased or contaminated animals into the facility. The type of transport container and the procedures for transportation and reception should follow the requirements for other mice. An animal protocol is required for any mice to be procured or bred in-house for feed. The mice should be euthanized prior to feeding.

- The qualifier ‘live’ should precede ‘feed’:
  - If mice are to be procured as LIVE feed...
  - An animal protocol is required for any mice to be procured LIVE and bred in-house for LIVE feed.

N) 4.4.3 Moving Mice within an Institution: ...Care should be taken to maintain the cage in a horizontal plane during transport.

- In addition to the placement of cages in a horizontal position, ventilation should be optimized (cages should not be stacked - a not uncommon observance in the field) and cages should be transported in such a manner that mitigates accidents, e.g. cage droppage and mitigates escape.

O) 5.0 Breeding: Record Keeping and Oversight: ...Records should be kept for the following...

- The enumerated list should include criteria for retirement from breeding. It is not an uncommon experience for vivarial personnel and veterinarians to witness attempts to breed reproductively senescent animals.

P) 5.4 Factors Affecting Reproduction – 5.4.1 Environmental Factors: ...Perfumes and other strong odorants should be avoided when working with mice in general.

- This is general practice and applies to housing and care of all mice (and other species). Accordingly it should not be buried within the breeding colony section. Might section 3 be appropriate?

Q) 5.6 Genotyping – The choice of method for obtaining genetic material should be based on 1) the aim to minimize pain and distress for the animals; 2) the amount of tissue needed, which will depend on the type of analysis required; and 3) whether a
suitable tissue sample can be obtained from the method used for identification of the animal (see Sections 5.2, “Identification of Breeding Colony Animals”, and 6.1, “Identification of Animals”). For a review of welfare concerns and potential refinements for methods of genotyping, see Appendix 4.

- Add: The genotyping methodology to be used must receive ACC approval and be described in an SOP.

R) 6.3 Food, Water & Bedding – 6.3.1 Food: ...Irradiated food should be used in SPF facilities...If food needs to be ground in-house, it should be prepared according to an institutional SOP (in an appropriate room using appropriate equipment).

- There is no mention of autoclavable food.

- There is a requirement to consult with the veterinary team to ensure that a proposed diet contains adequate nutrients (is a palatable, edible, nutritionally balanced) unless nutrient manipulation is the object of the research exercise. Diet proposals must be pre-approved by the ACC with veterinary consultation.

S) 6.3.2 Water: As noted in the CCAC guidelines: ...SPF mice may require specially treated water (e.g., acidified, chlorinated, reverse osmosis or UV irradiated) to prevent introduction of pathogens.

- Add ‘autoclaved’ to the enumerated list: (e.g. acidified, chlorinated, reverse osmosis, UV irradiated or autoclaved).

T) 6.3.3 Bedding and Nesting Materials – Guideline 17: Bedding and nesting materials must be non-toxic, produce a minimal amount of dust, be absorbent but not dehydrating for neonates, be inedible and consist of particles that are suited to the needs of mice.

- Add to Guideline 17 – and not contribute to harm or injury (e.g. limb entanglement, ocular lesions, other).

- The word ‘complex’ should be substituted for the word ‘better’ where better is used to describe nesting material quality. It is complexity that is key to effectiveness and more appropriately supports the recommendation for two types of nesting materials.

- The use of paper bedding is recommended on the one hand and yet cited as being susceptible to wetness from urine. Clarity is required.

- Paper and wood chips are edible – reconsider the wording e.g.: not harmful if ingested.
6.4 **Environmental Enrichment – Guideline 18:** Environmental enrichment should be a core consideration to enhance the welfare of mice.

- Enrichment must respect biosecurity and research protocols (e.g. fruit, vegetables, cereals). Enrichment modalities must be compatible with the intended research and be the product of consultation between researchers and the veterinary team. All modalities must receive ACC approval and be described in SOP’s.

- Enrichment sundries e.g. chew toys, should be discarded or replaced if they become worn and a welfare hazard.

6.7 **Cage Changing and Sanitation:** ...Cages need to be changed often enough to allow mice to have separate areas for living and sleeping and for soiling.

- Is this not a function of cage size and design rather than changing frequency? Clarification is required.

10.3.1 **Injections:** Substances to be administered should be non-irritating.

- Substances to be administered should not be irritating.

10.3.2 **Oral Dosing:** The smallest volume possible should be administered; optimally 5 ml/kg.

- Volume reduction is desirable and represents a standard change (from 20 ml/kg).

- Due consideration should be given to the effects of increased compound concentration (in the reduced volume) and consequences, whether adverse to the animal, e.g. gastric irritation or the operator, e.g. increased complexity of administration.

- A statement should be added regarding this change and established toxicology protocols.

10.4.1.2 **Retro-orbital bleeding** has been associated with negative animal welfare consequences and therefore should only be performed as a terminal procedure.

- ‘Terminal procedure’ should be qualified as follows: *Terminal procedure under anaesthesia or immediately following euthanasia.*

- It is not acceptable to inflict pain because the animal will be euthanized following this.
2) **10.6.2 Collecting Samples for Genotyping:** The sampling method should be the least invasive method that can provide the quantity and quality of tissue required for the particular genotyping method being used ... Toe clipping is discouraged, and is only permitted in particular situations with ACC approval where: 1) no other method can be used; 2) the mouse is very young (7 days old); and 3) only one doe is to be clipped.

- Toe clipping should be disallowed given technological advancements and available alternatives that are less mutilating, disabling to prehensile animals and their activities and thereby have less impact on animal welfare.

- If toe clipping is approved based on the inappropriateness of other methodologies, what happens when the mouse pup is 8 or more days old because the research technician or graduate student could not attend to this on day 7 (a not uncommon experience in the field). It follows then that an alternative methodology must be used. This being the case, then toe clipping is not acceptable in the first instance. An alternative methodology must be proposed and approved by the animal care committee when toe clipping is advanced as preferred methodology.

- Commentary on the impact of the amputation re. pain and disability and the experimental consequences of these need to be specifically addressed in the text – i.e. need for mindfulness of the impact of this genotyping method on experimental outcomes.

A.1) **10.11.1 Anesthesia:** When inhalants are not suitable, for safety or practical reasons, hypothermia (for mice less than 7 days old) should be considered in preference to injectable drugs (Danneman and Mandrell, 1997).

- The text should specify the target body temperature to achieve insensibility to pain and what methodology (dry ice, other) is optimal for this purpose.

- One can reasonably expect that the 7 day age maximum will be challenged in practice based on experience in the field. Accordingly an alternative methodology must be proposed and approved by the animal care committee when hypothermia is presented as the preferred choice of anaesthesia.

- General Comment – Drug dosage may need to be adjusted based on the animals’ circadian rhythm and time of day the anaesthesia is performed.

A.2) **10.12 Surgery:** Warm fluids should be administered to compensate for evaporation through open surgical wounds and blood loss, and to support blood pressure... Any procedures performed should be indicated on the cage card for cage-side assessment of
The mice. More detailed surgery logs should be kept by the researchers and made available to the veterinarian and to the ACC as needed.

- The maximum temperature for fluid warmth should be specified.
- The detailed surgical logs should be accessible to the veterinarian at the animal room level or electronically (respecting confidentiality) to be able to effectively assess and treat the animals as per the ACC-approved SOP for the study. The veterinarian should not have to ask for them.
- Good lighting is important however mouse eyes must be protected under high lighting conditions.

A.3) 10.13 Monitoring Post-procedural care and experimental endpoints – Guideline 27: Post-procedural care and monitoring must be planned based on the invasiveness of the procedure and the individual needs of the animals, and adapted to any unforeseen situations.

- This guideline should be revised as follows: Post-procedural care and monitoring, to include intervention, treatment, application of humane end-points or euthanasia, must be planned...
- In the text associated with this guideline, there is no mention of ACC and researcher approved care strategies to address complications arising from procedures and this is critical to post-procedural care and humane end-points.

A.4) 11.1 – Injection: ... However, intraperitoneal administration of irritating substances (e.g., pentobarbital) may provoke pain and has been shown to produce inconsistent outcomes in rats (Chisholm and Pang, 2016); hence, its use should be carefully evaluated and approved by the ACC.

- If intraperitoneal pentobarbital is selected for euthanasia, consider the addition of lidocaine to abrogate irritation-associated discomfort.

A.5) Appendices – Appendix 5 – Indicators of Disease: The format of this appendix could be improved for greater clarity.

- Death should be added as an indicator of disease as prey animals such as mice with protective conditioned responses (stoicism) may not exhibit clinical signs or behaviours suggesting disease. Often the first indication of a colony problem is unanticipated death(s).

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