AMPHIBIANS

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These frequently asked questions (FAQs) are intended to assist investigators, instructors, and members of animal care committees in the implementation of the *CCAC guidelines: Amphibians* (CCAC, 2021). FAQs provide general responses to comments and questions received by the CCAC during the external reviews of this guidelines document.

If you do not find the answer to your question here, do not hesitate to contact the CCAC and we will be pleased to provide assistance.

1. Do the guidelines cover amphibians in field studies?

The *CCAC guidelines: Amphibians* (CCAC, 2021) focuses on amphibians that are held in laboratory facilities or are brought into facilities from the wild. For studies involving amphibians in the wild, including short-term holding in the field, see the *CCAC guidelines on: the care and use of wildlife* (CCAC, 2003).

2. When is it safe to add amphibians to a new tank?

It is critical that tanks are commissioned before amphibians are introduced. This involves running a full battery of water quality tests for all variables described in Section 3.1.3.2, "Water Quality", to ensure they are within acceptable ranges. In addition, water that is treated with reverse osmosis or deionized needs to be allowed to age to reconstitute a beneficial microbial community.

3. What is the importance of the skin microbiome of amphibians and how can it be maintained?

The skin microbiome of amphibians is important in fighting off bacterial and fungal infections. To maintain a healthy skin microbiome, there needs to be a beneficial microbial community in the animal's immediate surroundings. This has implications for the housing environment and husbandry practices. It is important that the environment of amphibians be kept clean but not sterile. The rate of water change in enclosures for aquatic amphibians should be based on maintaining appropriate water quality to meet the animals' needs, which includes the development and maintenance of the skin microbiome.

4. CCAC guidelines documents now put greater emphasis on welfare assessment. What is expected of those working with amphibians?

Welfare assessment is a necessary component of all animal-based studies to ensure a good quality of life for the animals within the constraints of the study and for the quality of scientific data. The *CCAC guidelines: Animal welfare assessment* (CCAC, 2021) details the general requirements for all animals, and the *CCAC guidelines: Amphibians* (CCAC, 2021) builds on this foundation to focus on assessing the welfare of amphibians. Because this group of animals includes a wide range of species that collectively occupy very diverse habitats in nature, the guidelines provide general behaviours and physiological parameters to be used as a starting point. For example, behavioural indicators of welfare include changes to feeding behaviour, social interactions, and unexpected behavioural responses. The guidelines note that behaviours must be considered within the context of the animal's environment, as a particular change in behaviour may be indicative of varying stress levels, depending on the situation. For example, a diminished avoidance response could indicate lethargy, or it could mean that the frog is habituated to personnel. As well, activity levels can fluctuate in response to factors that do not necessarily relate to the welfare of an individual (e.g., seasonal changes). It is important that investigators are familiar with the behaviours and physiology of the species and individual animals they are working with so that they can detect changes that may have welfare implications.

5. There has been an ongoing debate in the scientific literature as to whether certain types of animals experience pain. How has this been addressed in these guidelines?

Amphibians have a well-developed nervous system with nociceptors and pathways for the perception and processing of noxious stimuli (Guénette et al., 2013). Nociception is transmitted to the central nervous system; however, there is limited information on whether these pathways reach the brain center, resulting in conscious perception of pain (Guénette et al., 2013). Due to the limitations of the evidence, the precautionary principle should be applied such that all necessary attempts are made to minimize potential suffering.

6. When are anesthetics and analgesics required for work with amphibians?

The guidelines state that anesthetics must be used in procedures where there is expected to be noxious stimuli and in experiments entailing extensive handling or manipulation with a reasonable expectation of trauma and physiological insult to the amphibian. There is considerable evidence for the use of a number of anesthetics on amphibians, and this is detailed in Section 10.10.1, "Anesthesia". There is currently less evidence available for the use of analgesics on amphibians. To acknowledge this situation while ensuring amphibians receive optimal care, the guideline in Section 10.10.2, "Analgesia", states "Following the precautionary principle, amphibians should be provided with analgesia for procedures that are likely to be painful, based on the best available scientific evidence." Analgesia is encouraged where relevant information can be found, and the inability of observers to recognize the nociceptive response or detect distress should not be justification for withholding analgesia. However, in the absence of relevant information on analgesics, there may be unknown side-effects for the animals and impacts on the research.