**Additional questions on the use of *Xenopus* *laevis* & *Xenopus* *tropicalis* overseas**

The expectations of the major UK public funding bodies for the use animals in bioscience research are set out in the document ‘[Responsibility in the Use of Animals in Bioscience Research’](https://www.nc3rs.org.uk/responsibility-use-animals-bioscience-research). Compliance with the principles in this document is a condition of receiving funds for animal research. Welfare standards consistent with the principles of UK legislation must be applied and maintained,whereverthe work is conducted. For further information, see [www.nc3rs.org.uk/use-animals-overseas](http://www.nc3rs.org.uk/use-animals-overseas)

Please confirm the following: (Y/N)

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| 1. The frogs have been bred and reared in captivity for scientific purposes. Use of *Xenopus* taken from the wild should be justified below. |  |
| 1. Enclosure sizes and space allocations meet or exceed those in Annex III to [Directive 2010/63/EU](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32010L0063) (Table 9.2). |  |
| 1. Frogs are housed in tanks free of sharp edges made from a non-leaching material, with opaque or at minimum translucent walls. |  |
| 1. **If this research utilises *Xenopus laevis***: Tank water is persistently monitored and maintained at a temperature range of 17 - 20oC (63-68F), pH of 7.5 - 9 and conductivity range of 1500 - 1600µS.   **If this research utilises *Xenopus tropicalis***: Tank water is persistently monitored and maintained at a temperature range of 24 - 27oC (75-81F), pH of 7.5 - 9 and conductivity range of 700 - 900µS.  **For both species**:Ammonium and nitrate levels are monitored at regular intervals and kept within safe limits |  |
| 1. Tank water is cleaned or replaced at appropriate intervals using methods designed to minimise disturbance and distress to the frogs and avoiding the use of cleaning products or disinfectants that risk harm to the animals. |  |
| 1. Frogs are housed in sized-matched groups to promote increased feeding behaviour, reduce fear responses and prevent cannibalism. |  |
| 1. Frogs are provided suitable, shaded refuges to hide in or under (e.g. PVC or ceramic tubes). |  |
| 1. Frogs are subject to an appropriate day/night light cycle, and the lighting used includes wavelengths within the UV spectrum. |  |
| 1. Frogs are identified using minimally invasive identification techniques. Use of invasive techniques such as toe clipping should be justified below. |  |
| 1. Appropriate measures are in place when handling and housing the animals to minimise the risk of infection. |  |
| 1. Appropriate, contemporary anaesthesia and/or analgesia is provided to minimise pain and distress. Any withholding of pain relief during painful procedures must be justified below. |  |
| 1. Surgery is performed using appropriate aseptic technique, the least invasive surgical approaches, and appropriate care is taken to prevent the animal drying out during surgery or drowning while recovering from anaesthesia. |  |
| 1. Animals are monitored with a frequency appropriate to keep pain and distress to a minimum, using welfare indicators (e.g excessive shedding, loss of appetite) and score sheets. |  |
| 1. Humane endpoints have been established for each experiment with the potential to cause moderate or severe harm, after consultation with the veterinarian and animal care staff, and implementation of these is recorded during the experiment. (Note the humane endpoint criteria may be requested by the funding body). |  |
| 1. The methods of humane killing are those recommended by the [AVMA (2013)](https://www.avma.org/KB/Policies/Documents/euthanasia.pdf) or permitted under [Directive 2010/63/EU](https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF) (Annex IV). |  |

Where there are deviations from the above, please explain below:

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