# Additional questions on the use of zebrafish outside of the UK

UK funders expect high standards in animal research regardless of geographical location. This includes meeting or exceeding UK standards of animal welfare, such as those set out in the UK Home Office [Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf) and the NC3Rs guidelines on the [Responsibility in the Use of Animals in Bioscience Research](https://www.nc3rs.org.uk/responsibility-use-animals-bioscience-research).

Applicants are responsible for obtaining sufficient information to accurately complete the sections below, this includes work that will be conducted by overseas collaborators or contracted to Contract Research Organisations (CROs).

For more information on this form, including guidance on how to complete it, visit the [NC3Rs website](https://nc3rs.org.uk/3rs-resources/peer-review-and-advice-service).

## Section 1. Checklist of additional questions

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| **The zebrafish on this study will be…** | **Yes/No/Not applicable (NA)** |
| 1. Bred and reared in captivity for scientific purposes.
 | Select |
| 1. [Socially housed in conditions that promote fish health and welfare](https://doi.org/10.1177/0023677219869037) including all of the following: a) separate housing for different life stages; b) age-appropriate stocking densities that allow daily visual inspection of fish within the tank (e.g. 10-30 larvae/litre for 5-30 dpf; 4-10 fish/l for >30 dpf); c) maintenance at a stable water temperature within the range of 24-29oC; d) lids covering all tanks.
 | Select |
| 1. Maintained in water with parameters falling within the bounds of the following ranges, taking into account that the potential toxicity of ammonia increases with pH: total ammonia (NH3/NH4) <0.1 mg/l; nitrite (NO2) <0.1 mg/l; nitrate (NO3) <25 mg/l; pH 6.5-8; conductivity 150-1700 µS/cm; general water hardness (dGH) between 3-8; dissolved O2 >4 mg/l and CO2 <20 mg/l. Chlorine is removed from tap water before use.
 | Select |
| 1. Maintained in stable environmental conditions and gradually acclimated to changes in water temperature and/or quality. Contingency plans are in place for maintaining stable environmental conditions in case of system breakdown or loss of power. When fish are held in static tanks for any purpose these are monitored closely to ensure that water quality and temperature do not reach levels outside of those given in rows 2 and 3 above.
 | Select |
| 1. Routinely fed 2-3 times a day (larval fish) or 1-3 times per day (adult fish) on a diet of formulated dry feed and/or pathogen-free live feed, ensuring the size of the feed is appropriate for the life stage of the fish.
 | Select |
| 1. Provided with environmental enrichment, including at least one of the following: a) gravel substrate or an image of gravel substrate; b) feeding of live prey; c) a refuge within the tank (e.g. synthetic plants or shelters).
 | Select |
| 1. Managed with appropriate biosecurity protocols including all of the following a) a quarantine system and health screening policy; b) bleaching of embryos before introduction to the main facility; c) hand nets designated to specific tanks/racks and sanitised between uses; d) a filtration system that includes a UV steriliser; e) [daily monitoring of fish health](https://doi.org/10.30802/AALAS-CM-22-000034).
 | Select |
| 1. Given anaesthesia and/or analgesia to [minimise pain](https://doi.org/10.1177/00236772231198733) and distress as advised by a veterinarian. *Select ‘No’ if the study is expected to cause pain beyond brief, mild discomfort and anaesthesia and/or analgesia will not be provided to alleviate this. Select ‘NA’ if animals will not undergo any procedures expected to cause pain beyond brief, mild discomfort. Note that fin clip/biopsy should be conducted under anaesthesia with perioperative analgesia.*
 | Select |
| 1. Operated on using a) aseptic technique, b) the least invasive surgical approaches and c) appropriate perioperative care (pre-operative medications and maintenance of body temperature). Assisted recovery/resuscitation is provided if necessary (e.g. through use of an aerated recovery tank). *Select ‘NA’ if no surgery will take place.*
 | Select |
| 1. Monitored using a method of [welfare assessment](https://www.nc3rs.org.uk/3rs-resources/welfare-assessment) that allows staff to take prompt and consistent action should a [humane endpoint](https://www.nc3rs.org.uk/3rs-resources/humane-endpoints) be reached. *Select ‘No’ if death will be used as an endpoint. Select ‘NA’ if this study will not cause harm, pain or distress to the animals. It is recommended that humane endpoints are established in consultation with the veterinarian and animal care staff and reviewed by the UK Animal Welfare and Ethical Review Body (AWERB) before the study commences.*
 | Select |
| 1. Monitored closely for emerging and harmful phenotypes if animals are genetically altered, with known harmful phenotypes being avoided. *Select ‘No’ if harmful phenotypes are considered scientifically justified and provide justification in Section 2.* Select ‘NA’ if *no genetically altered strains will be generated.*
 | Select |
| 1. Humanely killed using methods permitted for the species within the UK Home Office [Guidance on the operation of the Animals (Scientific Procedures) Act 1986](https://assets.publishing.service.gov.uk/media/65815e32ed3c3400133bfb07/Guidance_on_the_operation_of_ASPA_-_December_2023.pdf) (Appendix D). *Select ‘No’ if the method is not listed as appropriate method of humane killing of species within Appendix D of this guidance and use Section 2 to indicate the method of killing and provide the name of the guidelines that recommend the method. Select ‘NA’ if the animals will not be killed.*
 | Select |

## Section 2. Exceptions and additional information

For each item in *Section 1. Checklist of additional questions* where ‘No’ has been selected, provide brief information outlining:

* Why this study will require exceptions to the conditions outlined in the checklist (this should be a scientifically principled justification).
* What action will be taken to mitigate potential animal welfare issues related to this exception.

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## Section 3. Declaration

**I confirm that:**

* The information provided within *Section 1. Checklist of additional questions* has been verified by a representative at the institution where the animal work will take place. The representative has confirmed that the conditions outlined in this document will be maintained during the study. The details of the representative are:
	+ - Name:
		- Institution:
		- Job title:
		- Email address:
* The animal work taking place outside of the UK will be reviewed by a UK institution’s Animal Welfare and Ethical Review Body (AWERB) before the study commences and this document will be provided to the AWERB for their records. The details of the AWERB are:
	+ - Institution:
		- Committee/subcommittee name:

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| Principal applicant name:      Date:       |

\*If you are based at an institution outside of the UK without a UK partner institution and therefore this work cannot undergo review by a UK AWERB, check this box [ ]