Report on the outcomes of a Short-Term Scientific Mission[[1]](#footnote-1)

Action number: CA18221

Grantee name: Frances Kinross

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| **Details of the STSM**  Title: 2.1. Update of the database on amphibian ecotoxicological literature and review information on chronic endpoints indicative of reproductive effects, including quality assessment  Start and end date: 20/02/2023 to 31/03/2023 |
| **Description of the work carried out during the STSM**  Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section. |
| *(max. 500 words)*  The original aims of this STSM were to assess a range of reproductive endpoints for their sensitivity to pollutant exposure (“exposure biomarker”) as well as their potential importance for adversity in the context of successful reproduction (“effect biomarker”). In order to accurately report on the reliability of the available data, the second goal of this report was to define quality criteria against which reviewed studies can be assessed. However, upon review of the BCAT system developed by the host (Emily McVey) in person at CTGB and discussions between Frances Kinross and Emily McVey, it was decided that no amendments to the BCAT were needed as it was already fit for purpose.  It also became apparent that when considering adversity, there were no reproductive endpoints currently measured for chemicals risk assessment in amphibians that could be reliably linked to adversity. This is in part because the Larval Amphibian Growth and Development Assay (LAGDA: Table 1) has only been carried out a handful of times, and the other test guidelines which use amphibians as the test organism do not measure reproductive endpoints – i.e. the Amphibian Metamorphosis Assay (AMA) and the *Xenopus* Eleutheroembryo Transgenic Assay (XETA). Further, in the peer-review literature, where impacts on reproductive endpoints are reported, such as changes in sex ratio or in gonadal histopathology, linkages to adversity are either not reported or not well understood. For example, feminisation could arguably lead to increased populations, as females are typically the limiting sex in vertebrates due to the energy investment required for egg production.  However, one of the endpoints which is measured in AMA is body weight of the tadpoles on day 7 and 21 (Table 1). As it is known that size of tadpoles, and by extension size at metamorphosis, is inextricably linked to survival and reproductive success, body size (snout-vent length [SVL] and body mass) was chosen as an appropriate endpoint to assess adversity in the context of adverse effects on the reproductive potential of a population. In particular, as the AMA is regularly carried out and therefore much more data are availble for tadpole morphometrics compared to reproductive endpoitnts that are measured in the LAGDA.  Table 1. Primary (●) and optional (○) endpoints and timing of measurements in the AMA and the LAGDA according to OECD TG 231 (AMA) and 241 (LAGDA).    a Non-specific for thyroid.  b Non-specific for thyroid unless accompanied by significant thyroid histopathology  \* Phenotypic sex only (based on gonad morphology).  \*\* Overt and significant changes in apical endpoints indicating developmental acceleration or asynchrony may preclude the necessity to perform histopathological analysis of the thyroid glands. However, thyroid histology may be required by some regulatory authorities regardless of the apical responses.  Therefore, part of the aim of this STSM was to collect tadpole morphometric data from the AMA test guideline for a range of substances, to investigate the sensitivity of SVL, body mass and condition index (CI: weight/SVL) to chemicals exposure. In addition, we sought to compare this effect data with that collected as part of the Early Life Stage (ELS: TG210) fish assay, in order to determine whether effects on fish can be considered protective for amphibians. |
| **Description of the STSM main achievements and planned follow-up activities**  Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.  *(max. 500 words)*  Data on the AMA and ELS were collected for 6 substances (Table 2). Overall, there was no evidence that the morphological endpoints measured in the AMA provided a more sensitive indication for adversity compared to similar endpoints measured in sheepshead minnow (*Cyprinodon variegatus*) or rainbow trout (*Oncorhynchus mykiss*). Conversely, effects on the fish species recorded as part of the ELS test guideline were consistently more sensitive than for amphibians tested using the AMA – with just one substance of the six (substance E) found to cause a larger effect in *Xenopus* larvae compared to rainbow trout, though this was marginal and non-significant (slope: -1.6E-3 *versus* -1.8E-3). Further, the most sensitive endpoint was body weight (four out of six substances).  Table 2. Linear regression slope values for six substances (A-F) tested in the AMA and the ELS (either fathead minnow or brown trout or both). Values in bold are the most sensitive endpoint for each substance   |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Substance | AMA 7 | | | AMA 21 | | | Fathead | | | Trout | | | *P* | | SVL | WGT | CI | SVL | WGT | CI | SVL | WGT | CI | SVL | WGT | CI | | A | -5.2E-3 | -2.4E-4 | -8.7E-6 | 1.8E-4 | -9.1E-5 | -4.0E-6 | -4.7E-3 | **-2.8E-3** | 1.4E-4 | - | - | - | 0.001 | | B | -7.8E-4 | -3.6E-5 | -1.5E-6 |  |  |  | -1.8E-3 | **-1.6E-2** | -5.7E-4 | -6.7E-3 | -2.3E-2 | -6.4E-5 | < 0.0001 | | C | -1.9E-3 | 4.7E-3 | 3.2E-4 | 0.15 | 1.7E-2 | 4.2E-4 | - | - | - | -2.62 | **-31.1** | -0.54 | < 0.0001 | | D | -0.14 | -0.40 | -0.02 | -1.02 | -0.06 | -9.8E-4 | - | - | - | -8.06 | **-62.6** | -1.10 | 0.26 | | E | **-1.6E-3** | -1.1E-4 | -4.2E-6 | -1.5E-4 | -4.3E-4 | -1.4E-5 | - | - | - | 1.6E-4 | -1.8E-3 | -1.0E-4 | 0.99 | | F | 0.63 | 1.2E-2 | 5.5E-5 | -1.35 | 2.3E-2 | 3.3E-3 | **-0.19** | -0.15 | -3.0E3 | - | - | - | 0.82 |   SVL = snout-vent length; WGT = weight; CI = condition index (WGT/SVL). *P* value indicates extra sum of squares F test for testing the differences between regression slopes.              Figure 1. Linear correlations (Least Squares regression) for six substances (a-f). SVL\_7 = snout-vent length for *X. laevis* on day 7 of exposure; wgt\_7 = weight for *X. laevis* on day 7 of exposure; CI\_7 = condition index on day 7 of exposure for *X. laevis*; SVL\_21 = snout-vent length for *X. laevis* on day 21 of exposure; wgt\_21 = weight for *X. laevis* on day 21 of exposure; CI\_21 = condition index on day 21 of exposure for *X. laevis*; FH = sheepshead minnow; tr = ranbow trout.  Conclusions and future plans  There is no evidence from this study that amphibians are more sensitive than fish for detecting effects of test substances on morphometric endpoints. Indeed, the most sensitive endpoint was trout body weight, or where trout data were not available, sheepshead minnow body weight was the most sensitive endpoint. However, it was challenging to extract the AMA data as the final dossier documents for many substances have not been published (i.e. open access) to date and could not be obtained.  There are plans by Dr Kinross/McVey to extend this analysis to more substances in due course, as these become available, to investigate whether fish body weight remains as the most sensitive endpoint upon analysis of the data collected from a larger number of substances. |

1. This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant. [↑](#footnote-ref-1)