Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA18221

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Details of the STSM

Title: Can in vitro testing predict toxicity in vivo?

Start and end date: 15/06/2022 to 29/07/2022

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.

The main goal of the proposed STSM was to identify an appropriate *in vitro* methodology to be used as an alternative to *in vivo* experiments in the characterization of effects within the risk assessment frameworks of chemicals for amphibians. To attain this main goal, the effects of six variants of the surfactant sodium lauryl ether sulphate (SLES), each variant hold a different number of ethylene oxide groups, on the viability of two commercial cells lines of *Xenopus laevis* were assessed. The two cell lines that were studied originated from *X. laevis* and were: (i) A6 which are kidney epithelial cells obtained from an adult male of *X. laevis* and (ii) XTC-2 which are fibroblast cells obtained from tadpoles of the same species. Each cell line was exposed to serial concentrations of the SLES variants, and their viability was assessed through MTT Tetrazolium Assay (thiazolyl blue tetrazolium bromide) and resazurin. Adding to these *in vitro* assays with somatic cell lines, a trial *in vitro* assay was carried out by exposing spermatozoa (spz), freshly obtained from an adult male of *Pelophylax perezi*, to serial concentrations of two variants of SLES. Exposure occurred for 20 minutes, after this period the viability of spz was assessed through a live/dead kit working solution, consisting of Syto 9, propidium iodide and Ringer solutions.

Cell cultures of A6 and XTC-2 were grown on one-week time frame and consequently one test least one week despite that the surfactant effect was tested at 24h and 48h. As soon as there are no other studies about the effect of SLES on amphibian cell lines, I performed two trial tests to identify the range of SLES concentration where the effects appear on the cells. After the trial assays, I set up ten concentrations where the lowest concentration at 0.13 mg/L and the highest concentration at 1.32 mg/L. I carried out three independent assays, which were used as independent replicates, to calculate the concentration causing 10, 50 and 90% mortality (LC10, LC50, and LC90, respectively) for each variant of SLES. The following type of SLES were tested: SLE_0S, SLE_1S, SLE_4S, $SLE_{11}S$, $SLE_{30}S$, $SLE_{50}S$, where the number in the subscript indicates the number of the ethylene oxide units. Moreover, I compared the LCs between SLES as soon as it is hypothesized that the SLES with lower number of ethylene oxide

[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.

unit are more toxic compared with those with a higher number of ethylene oxide units. Because of unexpected loss of XTC-2 cells during the assay, I was forced to abandon the XTC-2 analysis due to time shortage.

Since LC50s are already available for embryos of *Pelophylax perezi*, in the future they will be compared with the LCs I computed for the A6 cell line in order to evaluate the adequacy to use these *in vitro* cell line assays to replace existing *in vivo* assays.

Finally, and adding to the mentioned above, during my STSM I had also the opportunity to collaborate in the maintenance of a culture of adults of *X. laevis*.

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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.

During my staying at the Department of Biology of the University of Aveiro, I could acquire competences on how to growth cell line cultures and perform cytotoxicity assays. Furthermore, I had the opportunity to learn new techniques of *X. laevis* adults maintaining and also new techniques about *X. laevis* reproduction in the laboratory.

Regarding A6 cell line, The MTT Tetrazolium Assay showed the lowest LC50 in SLE4S in both 24h and 48h (0.41 mg/L respectively 0.43 mg/L) while the higher LC50 was found in SLE50S for 24h and SLE1S for 48h (0.56 mg/L respectively 0.56 mg/L). The LC90 showed the lowest values for SLE0S in both 24h and 48h (0.57 mg/L respectively 0.55 mg/L) and the highest values for SLE50S in 24h and 48h (0.77 mg/L respectively 0.75 mg/L). While the LC50 and LC90 showed an ascending pattern from the SLES with low number of ethylene oxide units towards the SLES with a higher number of ethylene oxide units, LC10 has a higher variation between type of SLES. The results showed the lowest LC10 in 24h and 48h for SLE4 (0.2 mg/L respectively 0.29 mg/L). The highest LC10 was observed in SLE50S for 24h exposure (0.43 mg/L) respectively SLE1S in 48h of exposure (0.48 mg/L). The resazurin assay showed the lowest LCs for SLE0S in both 24 and 48h (LC10: 0.52 mg/L both 24 and 48h; LC25: 0.53 mg/L both 24 and 48h; LC50: 0.54 mg/L respectively 0.53 mg/L) while the highest concentration of LCs were found in SLE50S in both 24 and 48h (LC10: 0.79 mg/L respectively 0.73 mg/L; LC25: 0.81 mg/L respectively 0.75 mg/L; LC50: 0.95 mg/L respectively 0.78 mg/L). The LC90 was not estimated because the viability of the cells was higher than 10% in all cases and all concentrations. Overall, these results are in accordance with previous studies regarding the effect of SLES, considering the SLES with a low number low number of ethylene oxide more aggressive for other organisms (Martin et al., 2018; Fernandes et al., 2020). Moreover, further studies regarding the ecotoxicity of chemical compounds should be taken.

Spz exposed to SLE_1S (with 1 group of ethylene oxide) and $SLE_{50}S$ (with 50 groups of ethylene oxide), showed reduced viability at 0.3125 % v/v for SLE_1S and 0.03125% v/v for $SLE_{50}S$, i.e., SLE_1S was 10-fold more toxic than $SLE_{50}S$.

All the results obtained during this STSM will be published in a scientific journal. The results of the trial tests will be used as a starting point for further experimental design intended to find a solid *in vitro* approach for *in vivo* experiments regarding the amphibian risk assessment. This STSM strengthen collaboration between research institutions in Romania and Portugal and opens the opportunity for further joint research between this COST Partners.

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