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

Action number: CA18221

Grantee name: Simeon Lukanov

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| **Details of the STSM**  Title: Determination of pesticide effects on amphibians following dermal exposures during terrestrial stage  Start and end date: 16/07/2022 to 30/08/2022 |
| **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. |
| For the duration of the STSM, I was part of the Wildlife Toxicology research group of the Institute for Game and Wildlife Research (Instituto de Investigación en Recursos Cinegéticos, IREC) at University of Castilla-La Mancha, in Ciudad Real, Spain. The STSM objectives were: 1) investigate the toxicity of the active ingredients of different EU approved pesticide formulations on amphibian terrestrial stages and establish patterns of toxicity based on the properties of the substances; 2) determine the role that effects on skin, because of direct exposure, play on the toxicity of the different substances to terrestrial amphibians.  The main activity throughout my stay was to assist with the collection of tadpoles, their rearing to froglets (metamorphs) and the subsequent pesticide exposure experiments. Field trips to suitable ponds were undertaken on two occasions and dip netting was used to collect tadpoles in early stages of development (Gosner stage 26 or earlier). A total of 210 tadpoles were collected and added to the others already present at the laboratory facilities (around 550 in total). Tadpoles from different ponds were in different aquariums, designated by letters, and regularly counted to check for incidental deaths and development progress. Tadpoles were fed every other day with a fixed amount of standard fish food flakes, and all aquariums were cleaned two times per week. The water used for the aquariums was specially enriched in order to ensure optimal conditions, and was prepared at least a day before the cleaning using the FETAX medium (a combination of six different salts in strict proportions to 1 litre of water). After reaching Gosner stage 37, tadpoles were removed from their respective aquariums and housed in plastic boxes with aquatic and terrestrial part; each box contained 4-5 tadpoles from the same pond, which were not fed during this final stages. At completion of metamorphosis, each froglet was measured with a caliper, weighted, and placed in individual plastic box with entirely terrestrial setup, consisting of 100g of fine soil with added 30ml of FETAX-enriched water. During this stage, froglets were fed live mini-crickets two times per week (5 crickets for each froglet), and regularly checked for incidental deaths. Experiments commenced when there were at least 50 fully metamorphed froglets (10 for each test in step 3) and consisted of the following steps: 1) divide the frogs in pairs based on similar weight and size; 2) photograph each frog for individual identification; 3) spray with a fixed amount of FETAX-enriched water (control), acetone, and three different concentrations of the tested active ingredient (0.1x, 1x, 10x). Afterwards, each pair was housed in a plastic box with terrestrial setup, fed with mini-crickets and monitored daily. The preparation of the required active ingredients was done in sterile environment at the ecotoxicology laboratory of IREC.  In addition, another activity was to assist with tissue sample preparation for gas chromatography mass spectrometry analysis in the ecotoxicology laboratory of IREC. I assisted with running the samples through the standard analysis protocol, incl. multiple filtering, evaporation and resuspension. |
| **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.  The main achievement of the STSM is the successful initiation of experiments related to objective 1) investigate the toxicity of the active ingredients of different EU approved pesticide formulations on amphibian terrestrial stages and establish patterns of toxicity based on the properties of the substances. The number of metamorphs required to test the different active ingredients was reached and the experimental setup was working as planned. In the following weeks, all experiments should be concluded and the results analysed. Because of the initial shortage of tadpoles (due to altered and less successful breeding season for 2022), experiments began later than planned and work on STSM objective 2 (determine the role that effects on skin, because of direct exposure, play on the toxicity of the different substances to terrestrial amphibians) is yet to begin. However, results achieved until now still substantially contribute to the RCO related to continuously update and analyse the available information potentially useful to fill the gaps relative to the characterisation of pesticide exposure and effects on amphibians and reptiles. A number of substances (incl. fungicides such as Oxathiapiprolin) with unknown effect on amphibians were successfully tested and the results will allow to expand the knowledge on the effects of pesticide exposure. Depending on final results, which should be available by the end of the year, the experiments will likely produce several publications in high-impact factor journals.  In terms of CBO, the STSM provided me with helpful interdisciplinary training, adding a toxicology aspect to my ecology and behaviour background. The information I gained first-hand on exposure protocols and testing procedures would be very useful in conducting my own experiments with pesticides used in rice fields in Bulgaria (planned for 2023). More generally, I was introduced to novel amphibian husbandry techniques (i.e, the preparation of the FETAX medium), while providing some advice on fieldwork methods (i.e., use of funnel traps for tadpole capture instead on repeated visits for dip-netting, which I think are less effective). Through my work on this STSM, I established new research contacts and further developed existing ones, strengthening relations with my host at the IREC and other scientists from PERIAMAR who worked on related topics. Specifically, I developed a collaboration with a colleague form the University of Perugia who specializes in histology, and we plan to investigate the origin of a tumour found in a wild *Triturus ivanbureschi* specimen. The tumour, along with some preliminary microscope slides, is preserved in my laboratory at IBER-BAS and will be sent to Italy by the end of September 2022, so results on this collaboration are expected by the end of the year. With my hosts I discussed the possibility of adding behavioural aspect to toxicology research (e.g., avoidance behaviour), and we agreed in principle that their PhD students could help me and my students in a similar-styled STSM next year. |

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)