22-23/09/2022

# Subject │ Minutes of the Second General Meeting of COST Action CA18221: “PEsticide RIsk AssessMent for Amphibians and Reptiles”

1. **Welcome to participants and meeting presentation**

The participants (**Annex 1**) were welcomed by the Action Chair, Manuel Ortiz, and the local organiser, Katarzyna Kurek.

1. **Adoption of the agenda to participants and meeting presentation**

The agenda of the meeting (**Annex 2**) was adopted.

1. **Presentations of STSM outputs**

The holders of the STSM carried out so far during the actions, as well as other Action members currently involved in relevant projects, were invited to present their results and explain how the STSM contributed to achieve the Action objectives.

Samuel González-López, from the Spanish Institute of Game and Wildlife Research (IREC), presented the work “Determination of pesticide effects on amphibians following dermal exposures during terrestrial stage” as part of his ongoing PhD project and in the framework of the STSM conducted by Simeon Lukanov, from the Bulgarian Academy of Sciences, at the IREC. Results showed some mortality associated with overspray of juvenile *Pelophylax perezi* frogs with pesticides, but with the absence of dose-effect relationship.

Daniele Marini, from the University of Perugia, presented the work “Histological endpoints as tool to assess reproductive and skin toxicity in anurans exposed to pesticides”, as a compilation of the STSM he conducted at the University of Uppsala and a research stay he recently performed at the IREC. On the one hand, the work consisted of developing skin histological endpoints in juvenile *P. perezi* frogs and reproduction-related anatomical (nuptial pads, larynx…) and histological (gonads and ducts) endpoints in juvenile and adult *Xenopus laevis* that could be incorporated in characterization of toxic effects of chemicals.

Besta Dimitrova, from the Bulgarian Academy of Sciences, presented the work “Compilation of available toxicological data for biologically-based models to assess effects of pesticides on amphibians” as the result of the STSM she performed at the IREC. A database on amphibian and reptile toxicological literature that was compilated for an EFSA project[[1]](#footnote-1) was reviewed in search of data on repeated measures over time and dose-dependent responses that could potentially be useful for developing TKTD models. The potentially useful information is moderately abundant, although there is a clear lack relative to European species. It is clarified that the source database is publicly available, and that the part regarding reptiles has been recently updated through an STSM carried by Bárbara Santos at the National Institute of Biology of Slovenia. The convenience of updating it also for amphibians, possibly through another STSM, is agreed.

Cynthia Muñoz, from Radboud University, presented the work “Predicting maternal transfer of pesticides in reptiles based on pollutant molecular structure” as the result of the STSM she performed at the University Claude Bernard of Lyon. A model to predict maternal transfer based on reptile data was developed. The potential application of this model to risk assessment is discussed.

Sebastian Topliceanu, from the Ovidius University of Constanta, presented the work “Can in vitro testing predict toxicity in vivo?” as the result of the STSM he performed at the University of Aveiro. The potential of results collected in vitro to reflect effects on the whole organisms was discussed. The convenience of running comparative analyses with in vivo data was agreed as a measure to explore the extrapolation potential of *in vitro* testing.

Raluca Băncilă, from the Institute of Speleology Emil Racovita, presented the work “Distribution of amphibians and reptiles in agricultural landscape across Europe” as the results of the STSMs that she and Matteo Lattuada conducted at the University of Porto. The patterns of distribution of amphibians and reptiles across Europe in different types of agricultural uses and at three different scales were reviewed. A pattern for increased species richness in areas with increased crop heterogenicity is observed. The correlation among the different scales and the appropriateness of using one or another for risk assessment purposes (i.e. identification of potentially exposed species) is discussed.

Sabina Vlad, from the Ovidius University of Constanta, presented the work “Building a database linking life history traits and the response to agrochemicals in European amphibians and reptiles” as the result of the STSM that she conducted at CIBIO-University of Porto and what Alexandra Telea conducted at IREC. The output of these STSM is a life history trait database including 18 amphibian and 30 saurian species, which could become useful for identification of focal species.

Enerit Sacdanaku, from the University of Tirana, presented the work “Extended review of the potential use of ecological mesocosms in herpetofauna studies and their application to ERA of pesticides” as the result of the STSM that he conducted at CIBIO-University of Porto. After that, Miruna G. Vizireanu, from the Ovidius University of Constanta, presented the work “Feasibility of ecological mesocosm to environmental risk assessment (ERA) of pesticides on reptiles. A pilot test with wall lizards” as the results of the STSMs that she and Geanina Fanaru, from the same University, conducted at CIBIO-University of Porto. All these STSMs together served to design and test a mesocosm test of potential applicability for testing sensitivity of lizards to pesticides in an integrated environment.

Giulia Simbula, from the University of Rome 3, presented the work “Telemetry study on lizards *Lacerta agilis*” as the result of the STSM she conducted at RIFCON. The study served to evaluate the use of vineyards as a habitat for lizards and the frequency of occupancy of these habitats compared to off-crop areas.

1. **Presentation of the mind map-based paper on the risk assessment scheme**

The leaders of Working Group 3, Annette Aldrich, and Working Group 1, Andreas Focks, presented the project of preparing a paper based on the mind map on the risk assessment scheme that was elaborated in previous meetings. They also presented the inputs that were received from PERIAMAR members about the ongoing projects and how these can contribute to solve the questions reflected in the different mind map branches. It was agreed that the mind map was complete and that no more branches were, in general, needed. Only the section on grouping PPP by application system was suggested to be moved to the exposure characterization branch.

The provided inputs were reviewed. The main discussed items included:

* Focal species definition: the databases have been compiled (see presentation by Sabinal Vlad in agenda item 2) and now an STSM can help to select focal species, or generic (non-real) focal species.
* Translate and communicate Risk Mitigation Measures to farmers. Annette explained her project on communicating to farmers. Progress will be made next month through a dedicated workshop (not part of PERIAMAR). The importance of communication was stressed; Silvia Pieper suggested including a dissemination activity in this context.
* Consider innovative application technologies. Nobody provided inputs on this, but there is agreement on the importance of testing innovative technologies (e.g. precision farming) and evaluate specifically how these can influence amphibians or reptiles.
* Biological monitoring of pathogens. Manuel Ortiz explained that the AMPHIDEB project includes an objective consisting of monitoring the distribution of chytrid fungi and the fungus responsible for ophidiomycosis in snakes in Italy and in Spain, and that some environmental parameters (including pollutant loads in aquatic environments) are being measured to correlate with the presence of those pathogens. Emily McVey suggested including as an additional environmental correlate the presence of microorganisms that are potential competitors with fungicides. The problem is that the characterization of these pathogens seems to be well developed for soils but not for sediments, whereas the monitored fungi are essentially aquatic.
* Valentin Mingo explained the development of a terrestrial uptake model for dermal exposure. It is a generic model for anurans based on publicly available body burden data and considers calculation of internal exposure. The goal is to extrapolate the internal dose resulting from pesticide uptake from contaminated soil. Valentin explained the different stages of the model development. Ideally it should be able to make predictions on dermal exposure based on physico-chemical properties of the substances. The model is built on many assumptions, but all of these have some support.
* Frank Pasmans (connected online) explained some details about their project on agricultural contaminants in amphibian breeding waters: impact on dynamics and anti-Bs/Bd activity of aquatic micropredator communities. The project should contribute to some of the mind map branches, but not necessarily to that where it was flagged. The project details will be reviewed and it will be allocated in the branch where it is considered more relevant.
* Addressing (chronic) sublethal toxicity. Silvia Pieper stressed the necessity of getting a better understanding about how chronic toxicity impact reproduction. A discussion follows about the chances of establishing assessment factors for reproductive endpoints based on surrogate taxa. Some opinions reflected reluctancy due to lack of data for amphibians or reptiles. It is agreed to postpone this item and addressed it as part of the Working Group discussion in agenda item 5.

Some inputs were not reviewed due to the lack of time, but input providers will be addressed in case it is considered necessary.

The WG leaders encouraged people to keep sending contributions to fill the gaps in the mind map. With all these contributions, and once clarified the current ones, a first draft of the paper will be elaborated.

1. **Review of communication strategy and discussion on next steps**

The Science Communication Coordinator, Anamarija Žagar, presented the information on communication strategy within the action, including the composition of the Science Communication Committee, dissemination and communication objectives of the action, status of communication channels, current version of the communication strategy. We have not uploaded the Science Communication Strategy to e-cost; it would be good to review the current version and upload it before the end of this Grant Period, assuming that it can still be amended afterwards.

It is proposed to have an STSM focused on risk communication in the next GP. We need, however, to identify who can host and who can hold such an STSM.

The fact that we have not implemented any measure to reach farmers was stressed. In this context, it is important to know what to communicate, since now we do not have any final output. Frances Orton suggested contacting some specialists in communication with farmers she has liaised in Scotland. These specialists (or others from somewhere else) could help the Action to gain skills in how to communicate with farmers. It is agreed that a Virtual Mobility Grant can be proposed for the next GP to gain communication skills in this context.

On the other hand, communication must be on both directions, so it is also important to test opinions from farmers about our activities. There can be stakeholder groups or surveys. The Science Communication Committee will work in how to address this issue of approaching farmers.

1. **Proposal of a Risk Assessment strategy for amphibians and reptiles**

The co-leader of WG4, Emily McVey, explained that, since a fully comprehensive risk assessment including all important factors would require significantly more time and research and development than what is available in PERIAMAR, a “fit-for-purpose” scheme should be developed to achieve better protection in the very near future. This scheme will rely on currently available tools and data. It is assumed that this would be imperfect, but it would serve to provide some protection and also to stimulate further progress to improve the scheme.

The fit-for-purpose scheme should preferably inform at the very least on the “worst case” substances, inform risk managers on the need for mitigation, and identify the specific areas needing from further development and research. The longer-term goal of a more perfect scheme (i.e., more data-driven, incorporating better use of additional modelling and monitoring) should be the eventual goal, and key points for knowledge and technological development, as well as possible RA models for this improved version, should be identified as well.

The proposal is structured in three steps:

1. Identify specific protection goals.

These are advice and proposals; risk managers in the end decide upon the protection goal, however a RA can also not be “built” without them. The protection goals are envisioned as proposed by WG4 (with input from other WGs).

1. Define potential mitigation options

Mitigation options should be directly linked to quantitative risk assessment (e.g., drift reduction) and/or to other laws and policies (e.g., habitats directive, water framework directive, European Green Deal, CAP, etc.).

1. Develop the “fit-for-purpose” Amphibian and Reptile Risk Assessment, including:
* Effect assessment: additional *in vivo* tests should be avoided, hence it is important to use available endpoint(s) for determining and calibrating extrapolation/assessment factors. The tools for this would be an analysis of the reviews which have been performed already and develop extrapolation options based on (i) correlations of sensitivity with substance characteristics; (ii) comparison of endpoints between existing fish, mammal or bird endpoints and endpoints in amphibians and reptiles, (iii) allometric relationships between groups in toxicological sensitivity.
* Exposure assessment in the terrestrial environment. The environmental fate of pesticides in matrices relevant for amphibians and reptiles can be obtained from current fate models that estimate PPP concentrations in water (surface, runoff, pore), sediment, soil, and air.
* Internal exposure. A simple single compartment exposure model with worst-case kinetics seems the best initial approach. This can be extracted from existing dermal exposure models (e.g. TIM from US EPA). WG3 is envisioned to work (more) on this.

Once the scheme is designed, the experienced assessors from WG4 will test the “fit-for-purpose” amphibian and reptile RA as far as its ability to (i) appropriately differentiate low-risk substances in the first tier, and (ii) potentially differentiate substances which can be grouped by properties based on the tier 1 RA.

1. **Status of the Working Groups progress and planning of activities for Grant Period 4**

Following the identification of the main discussion points having come up during the previous agenda items, it is proposed to separate the group into three Working Group discussions:

* Identification of chronic toxicity endpoints and possibilities for extrapolation from other taxa. Members of WGs 1 and 3 will join this discussion.
* Incorporation of modelling into risk assessment. Members of WGs 1 and 2 will join this discussion.
* Definition of field-based aspects, including focal species, exposure refinement, monitoring and risk mitigation measures. Members of WGs 2 and 3 will join this discussion.

Members of WG4 will join the three discussions.

At the end of the separate discussions, the conclusions from each group were presented.

Emily McVey presented the conclusions of the first discussion group. It is proposed to have two to three STSM to address the following topics: (i) update the database on amphibian ecotoxicological literature and review information on chronic endpoints indicative of reproductive effects, including quality assessment of the studies and extraction of endpoints (values) for specific substances; (ii) extract information on ecotoxicological data in vertebrates from registration dossiers for specific substances, to use for comparison with the amphibian and reptile data; and (iii) complete identification of endpoints potentially useful for extrapolation and calculation of assessment factors. It is unclear whether the last one can be separated, as it will depend upon how much is accomplished in the first to STSMs, but in the interest of being prepared it is initially proposed as a separate topic. The results of these STSMs will serve as the basis for discussion in two meetings: one joint meeting of WGs 3 and 4 for determination of calibration factors, and another joint meeting of WGs 1 and 4 for feeding back on data gaps necessary to refine assessment factor calculations and/or advise development of additional options for effect assessment in the future.

Andreas Focks presented the conclusions of the second discussion group. It is proposed to have a dedicated workshop on the incorporation of modelling in amphibian and reptile risk assessment, and four STSM to address topics like: (i) make the alligator model useful for sand lizards; (ii) progress in ALMaSS modelling for the great crested newt; (iii) develop fate modelling combining aquatic and terrestrial environments; and (iv) use internal exposure to combine different exposure routes combined with statistical modelling.

Annette Aldrich and Valentin Mingo presented the conclusions of the third discussion group. It is proposed to have a workshop or meeting to address the possibilities of designing amphibian and reptile monitoring for post-registration assessment. Identification of focal species needs to be addressed through the analysis of the compiled databases on life history traits. In addition, two STSMs are proposed to address the question on risk mitigation, one on the identification of mitigation measures applicable to amphibians and reptiles from a habitat/environment perspective and another one on the legal applicability of risk mitigation measures for the specific case of amphibians and reptiles.

The proposals were registered for discussion within the MC meeting and eventual incorporation into the Work Budget Plan for the next Grant Period.

1. **Closing**

The Action Chair acknowledges the participation of all meeting attendants and thanks Katarzyna Kurek and the Institute for Nature Conservation for the organization of the meeting. It is reminded that all participants in the General Meetings that are not MC members or appointed substitutes are invited to stay for the MC meeting as hearing persons.

## **LIST OF ANNEXES**

**Annex 1 – List of Participants**

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**Annex 2 – Agenda**



1. <https://doi.org/10.2903/sp.efsa.2017.EN-1251> [↑](#footnote-ref-1)